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ABBREVIATIONS OF TITLES

Abbreviation	*Name of Periodical*
Agri. J. India	Agricultural Journal of India.
Annalen	Justus Liebig's Annalen der Chemie.
Ann. Appl. Biol.	Annals of Applied Biology.
Ann. Chim. Analyt.	Annales de Chimie analytique et de Chimie appliquée.
Ann. Chim. Phys.	Annales de Chimie.
Arch. Pharm.	Archiv der Pharmazie.
Atti R. Accad. Lincei	Atti della Reale Accademia Nazionale dei Lincei.
Ber.	Berichte der deutschen chemischen Gesellschaft.
Biochem. J.	Biochemical Journal.
Biochem. Z.	Biochemische Zeitschrift.
Bull. Agri. Res. Inst. Pusa	Bulletin of the Agricultural Research Institute, Pusa.
Bull. Imp. Inst.	Bulletin of the Imperial Institute.
Bull. Soc. chim.	Bulletin de la Société chimique de France.
Centr. Bakt.	Zentralblatt für Bakteriologie.
Chem. Zentr.	Chemisches Zentralblatt.
Compt. rend.	Comptes rendus hebdomadaires des Scéances de l'Académie des Sciences.
Compt. rend. Soc. Biol.	Comptes rendus hebdomadaires des Scéances de la Société de Biologie.
Deut. landw. Rundschau	Deutsche landwirthschaftliche Rundschau.
Eng. News Rec.	Engineering News Record.
Gazzetta	Gazzetta chimica italiana.
Imp. Gaz. India	Imperial Gazetteer, India.
Indian For. Records	Indian Forest Records.
J. Agric. Res.	Journal of Agricultural Research.
J. Agr. Sci.	Journal of Agricultural Science.
J. Amer. Chem. Soc.	Journal of the American Chemical Society.
J. Bact.	Journal of Pathology and Bacteriology.
J. Biol. Chem.	Journal of Biological Chemistry.
J. Chem. Soc.	Journal of the Chemical Society.
J. C. S.	Journal of the Chemical Society.
J. Gen. Physiol.	Journal of General Physiology.
J. Ind. Eng. Chem.	Journal of Industrial and Engineering Chemistry.
J. Indian Chem. Soc.	Journal of the Indian Chemical Society.

J. Indian Inst. Sci.	Journal of the Indian Institute of Science.
J. Landw.	Journal für Landwirtschaft.
J. Physical Chem.	Journal of Physical Chemistry.
J. pr. Chem.	Journal für praktische Chemie.
J. S. C. I.	Journal of the Society of Chemical Industry.
J. Soc. Chem. Ind.	Journal of the Society of Chemical Industry.
Kolloid-Z.	Kolloid-Zeitschrift.
Monatsh.	Monatshefte für Chemie und verwandte Teile ander Wissenschaften.
Oesterr. Chem.-Ztg.	Oesterreichische Chemiker-Zeitung.
Pharm. Zentr.	Pharmazeutische Zentralhalle.
Phil. Mag.	Philosophical Magazine.
Proc. Chem. Eng. Group	Proceedings of the Chemical Engineering Group: Society of Chemical Industry.
Proc. C. S.	Proceedings of the Chemical Society.
Proc. K. Akad. Wetensch.	Koninklijke Akademie van Wetenschappen te Amsterdam. Proceedings (English version).
Proc. Roy. Soc.	Proceedings of the Royal Society.
Quart. Jour. Chem. Soc.	Quarterly Journal of the Chemical Society.
Rec. trav. chim.	Recueil des travaux chimiques des Pays-Bas.
Sitzungsber. Akad. Wiss. Wien	Sitzungsberichte der Akademie der Wissenschaften zu Wien.
Sitzungsber. preuss. Akad. Wiss.	Sitzungsberichte der preussischen Akademie der Wissenschaften zu Berlin.
Soil Sci.	Soil Science.
Trans. Faraday Soc.	Transactions of the Faraday Society.
U. S. Dept. Agric. Bull.	Bulletin of the United States Department of Agriculture.
Woch. Brau.	Wochenschrift für Brauerei.
Z. Biol.	Biologisches Zentralblatt.
Z. Elektrochem.	Zeitschrift für Elektrochemie.
Z. physical. Chem.	Zeitschrift für physikalische Chemie, Stöchiometrie und Verwandtschaftslehre.
Z. physiol. Chem.	Hoppe-Seyler's Zeitschrift für physiologische Chemie.
Z. Ver. deut. Zucker-Ind.	Zeitschrift des Vereins der deutschen Zucker-Industrie.

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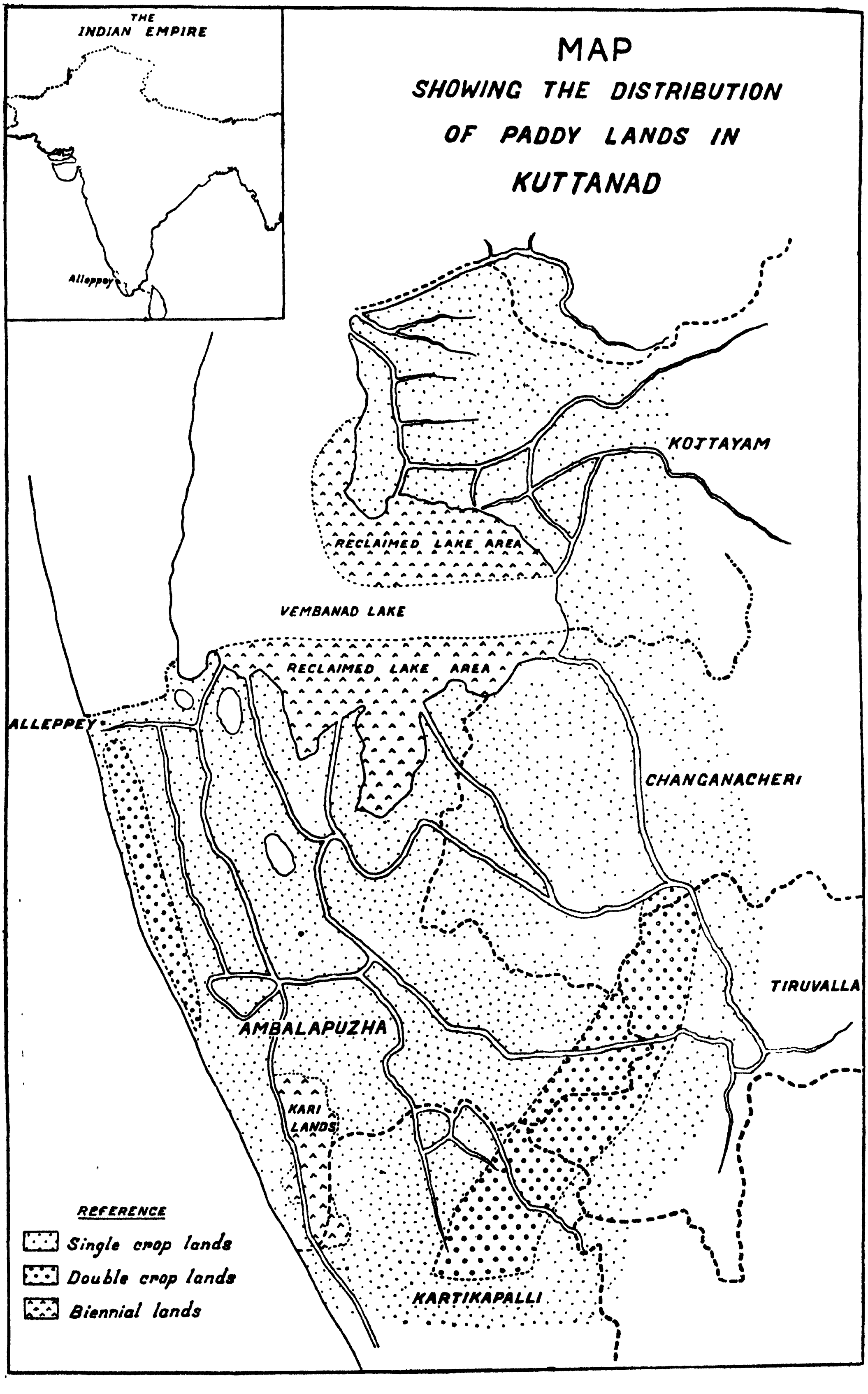

MAP SHOWING THE DISTRIBUTION OF PADDY LANDS IN KUTTANAD

SOME PECULIAR LOW-LYING SOILS OF CENTRAL TRAVANCORE.

By. T. R. Narayana Pillai and V. Subrahmanyan.

There is evidence that the area popularly known as *Kuttanad* was once a part of the Arabian Sea (K. Parameswaran Pillai, *Dept. of Agr. Travancore*, *Bull.*, 1924, 9). The land lies below sea-level, receives a high rainfall of about 150 inches and is subject to flooding by its numerous rivers and the sea. The slope is generally from east to west; and depending on locality, or farming enterprise, the cultivable area is under paddy (*a*) twice, or (*b*) once in the year, or (*c*) once in two years. Part of (*c*) is land reclaimed from a lake (*Vembanad*) and part from a marshy region (*Kari* land) which is peaty, and carries a characteristic vegetation.

Only on (*a*) is any crop raised during the monsoon months, June to October. The seeds are broadcasted during April and May when the land is generally dry. The rains begin soon after and the whole area passes under water and remains so till October, when the paddy is ready for harvest. If the opening rains are sudden and heavy, they uproot the plants and the crop fails entirely; but, if slow and steady, the seedlings withstand the flooding even if the depth of water exceeds 4 feet. The harvest is generally reaped when the land is under about 3 feet of water, and a yield of 1,200–1,500 lbs. of paddy per acre is obtained.

For the second crop on (*a*) and the only one on (*b*) and (*c*) the cultivation, which is begun either in late November or early December, is the same as elsewhere in the tropics with the following differences: (*a*) Shallow parts of the lake are reclaimed every alternate season by partitioning with high earthen walls and pumping out water into the main part of the lake until the level falls enough to permit cultivation; (*b*) The soil being wet and sticky, very little ploughing is possible; (*c*) Seeds are previously germinated and sown broadcast; (*d*) Owing to danger of early rains and consequent difficulty in drying, the straw is not cut but allowed to decompose in the soil. A yield of about 1,000 lbs. of paddy per acre is obtained.

Though Kuttanad remains one of the most fertile areas in the State, its yields are fast diminishing owing to disabilities such as (*a*) difficulties in cultivation, (*b*) unsuitedness of most chemical fertilisers, (*c*) danger of inundation with salt water, (*d*) decomposition of straw and sundry vegetation, (*e*) soil acidity and sickness, (*f*) plant diseases, and (*g*) visitation by grasshoppers and other insect pests.

A study of the soils was undertaken with a view to (*a*) determining the effect of the peculiar environment on their relative composition and character and (*b*) suggesting means for amelioration, and improvement of their yields. One of the authors (T. R. N. P.) toured the area in December, 1927; the collected specimens were air-dried and brought to Bangalore for examination.

EXPERIMENTAL.

Mechanical composition was determined by Robinson's pipette method as adopted by the Agricultural Education Association (*J. Agr. Sci.*, 1926, **16**, 123). The results have been recorded in Table I.

Although a number of soils were examined, only representative figures have been recorded in this and the following tables to clarify the main issues. Thus, A represents the average double-crop land; B, single-crop land; C, D and E, lake-area unreclaimed, or reclaimed 6 and 35 years ago respectively; also F, G and H, *Kari* land unreclaimed, and reclaimed about 6 and 40 years ago respectively. Where, however, differences between individual specimens of the same type exceeded those warranted by normal distribution, the maxima and minima of the ranges concerned have been recorded. Except when mentioned otherwise, the figures relate to surface soils only.

TABLE I.

Soil	Coarse sand	Fine sand	Silt	Fine silt	Clay	Hyg. moisture	Loss on ignition
A	7·95	17·32	9·08	17·20	22·92	9·54	14·63
B	7·09	20·23	9·06	16·50	26·13	9·33	10·42
C	2·34	39·54	8·25	11·50	19·00	6·49	9·41
D	2·77	47·20	7·13	9·88	15·50	4·65	8·82
E	20·70	37·60	4·50	7·50	15·75	4·63	7·70
F	14·07	0·55	0·75	7·75	11·25	27·90	33·10
G	5·05	12·00	8·00	15·50	28·25	11·65	17·20
H	22·90	21·35	10·00	14·50	13·75	8·60	6·10

The mechanical compositions of the average single and double-crop lands are similar, both being essentially light soils. The higher organic matter content of the double-crop land is probably due to the larger quantity of straw and other plant residues decomposing in it; but it does not appear to have affected the moisture retaining property which is nearly the same in both types of soil.

The lake-area is more sandy and contains less silt and clay than the other cultivated lands of Kuttanad. The portions under cultivation contain more of the coarser fractions than the unreclaimed ones, probably because the flood-water containing a large part of the lighter fractions is frequently pumped out. Since water is always plentiful, these soils are not likely to suffer from drought, although highly porous ; but their composition suggests that they require considerable amounts of organic manures to replace the plant nutrients removed by flooding.

The remarkable differences between the three types of *Kari* lands should be attributed to cultivation, which prevents stagnation, facilitates proper aeration and provides adequate drainage. Whereas the unreclaimed portions are sticky, and contain considerable quantities of visible undecomposed plant residues which smoke and burn on ignition, the reclaimed areas are porous and contain only traces of undecomposed organic matter. The marked differences in composition between the wild and the reclaimed areas indicate that with the progress of cultivation the whole of *Kari* land can be brought to normal conditions except where proper drainage is impossible. The increased proportion of coarser fractions observed in the cultivated areas is probably due to (*a*) diminution of organic matter which, as observed on peat soils, appears to have become oxidised readily on being brought under cultivation and (*b*) draining of flood-water as in the lake-area.

The mechanical composition does not throw any light on the moisture or air-relations of the soils, because the latter (*a*) receive a high rainfall, (*b*) are almost always under water and (*c*) contain high percentages of organic matter, all of which factors modify greatly the physical properties of the soils concerned.

The mechanical composition of the sub-soils (9in.–3ft.) follows nearly the same order as that of the surface (Table II). Because they

TABLE II.

Soil	Coarse sand	Fine sand	Silt	Fine silt	Clay	Hyg. moisture	Loss on ignition
A	9·75	14·30	7·88	16·25	32·50	6·92	11·01
B	4·6	24·66	8·44	13·88	27·44	8·26	10·21
C	0·78	42·10	9·00	7·25	20·25	7·52	9·81
D	1·71	24·79	20·75	17·75	12·13	8·63	6·16
E	17·57	48·92	3·25	7·00	12·00	3·00	5·80
G	3·45	15·46	6·75	15·25	26·00	10·28	16·94
H	27·35	19·28	7·50	13·25	14·25	6·30	6·75

are not much affected by floods, they contain generally more of the finer and less of the coarser fractions than the surface soils. The plant residues occur largely in the surface soils and hence the sub-soils generally contain less organic matter and hygroscopic moisture than the former.

Saturation capacities for water were determined by Hilgard's method (cited from Hall, The Soil, 1921, 73), and were A, 66·15; B, 53·78; C, 55·04; D, 51·94; and E, 63·96 per cent. respectively. F, G and H absorbed no water even after standing for 24 hours. On further examination, it was observed that those soils contained a wax-like substance coating the soil particles and rendering them impermeable to water; investigation of this material is in progress. After its removal with ether, the soils absorbed water readily. The presence of the wax accounted also for (*a*) the very high percentage of hygroscopic moisture observed in F, the waxy film having rendered proper drying impossible by mere exposure to air, (*b*) the continued wilting of seedlings generally observed in the *Kari* lands even after a rain if preceded by drought. Its persistent occurrence in the soil forty years after reclamation suggests that, although other forms of organic matter are decomposed readily in the cultivated soil, the wax is highly resistant and causes considerable damage to the crop.

Saturation capacities of the other soils did not bear any direct relation to their mechanical composition. As in the *Kari* soils, however, it is possible that the nature rather than the quantity of organic matter may have influenced their water-absorbing properties.

TABLE III.

Soil	Available phosphorus as P_2O_5	Available potash as K_2O	Total nitrogen
A	135	14	2600
B	29	48	1580
C	13	120	1200
D	42	103	1050
E	83	100	400
F	210	30	2500
G	40	411	3900
H	27	10	1200

Nitrogen, Potassium and Phosphorus.

K. P. Pillai's figures (*loc. cit.*) showed that the total potash and phosphoric acid of the soils varied with locality. Since his figures for available phosphoric acid and potash were not complete, determinations were conducted on the different soils by Dyer's method (*J. C. S.*, 1894, **65**, 115). Total nitrogen was determined by the Kjeldahl method (Chemists' Year Book, 1927, 806) and nitrates by the phenoldisulphonic acid method (Harper, *J. Ind. Eng. Chem.*, 1924, **16**, 180) ; nitrates were absent. The results in parts per million (Table III) showed that all the cultivated soils excepting the double-crop lands were highly deficient in available phosphorus : but since even the latter become depleted with successive cropping, it may be inferred that Kuttanad soils will respond to phosphorus in an available form. The application of a suitable phosphatic manure will also (*a*) promote better root development thereby enabling the plants to withstand the heavy early rains, and (*b*) bring them to early harvest and thus greatly minimise the crop-failures so frequently experienced in Kuttanad.

Not all the soils were deficient in available potash. The double-crop and reclaimed *Kari* lands were poor and will respond to potash; but the single-crop and the lake-areas contained sufficient amounts to last for some years.

With exception of those from the lake-area, none of the soils were deficient in total nitrogen, but it is still possible that the major part is unavailable. Since there is no adequate technique for determining the availability of nitrogen in the soil, it will be necessary to carry out field trials, and to determine from the plant-growth and crop-yield whether the different types of soil will benefit by application of nitrogenous fertilisers.

The entire absence of nitrates from the soils must be due to (*a*) leaching of nitrates during monsoon months, (*b*) hindrance to nitrification by waterlogging (Subrahmanyan, *J. Agr. Sci.*, 1927, **17**, 429) and high percentage of soil moisture and (*c*) 'locking up' by micro-organisms in the decomposition of straw and other plant residues which are allowed to rot in the soil. Absence of nitrates should not, however, lower the agricultural value of the soil, because (*a*) the rice-plant responds more effectively to ammoniacal fertilisers than to nitrates and (*b*) nitrates, if present in more than minimal amounts, are reduced to nitrites, which are highly toxic to the rice-plant.

REACTION, EXCHANGEABLE CALCIUM AND LIME REQUIREMENT.

The hydrogen-ion concentration of the soils was determined by Gillespie's method (*Soil Sci.*, 1920, **9**, 115), exchangeable calcium by Hissink's method (Chemists Year Book, 1927, 811) and lime requirement by the Hutchinson and McLennan method (*J. Agr. Sci.*, 1915, **7**, 75). The results (Table IV) showed that although most of the soils were slightly acid, their reactions, except in the case of the unreclaimed *Kari* land, were not such as would seriously affect plant growth. The reactions of the single-crop and the lake-area soils varied with locality and depended probably on the extent of (*a*) liming and (*b*) cultivation they received.

TABLE IV.

Soil	P_H	Lime requirement Gms. CaO per 100 gms. soil	Exchangeable Ca Gms. CaO per 100 gms. soil
A	6·9–7·1	0·200	0·083
B	6·7-7·3	0·169	0·097
C	7 3	0·078	0·183
D	6·4–7·5	0·142–0·261	0·929–0·280
E	6·9	0·140	0·131
F	5·9	0·565	0·128
G	6·5	0·414	0·232
H	6·9	0·140	0·083

The acidity of the soils appears to be due to organic acids formed during incomplete oxidation of straw and other plant residues which decomposed in them. It was high in the unreclaimed *Kari* soil where undecomposed matter had accumulated in the marshes for years. The advance of cultivation by providing adequate drainage and aeration appears to have caused the organic acids to be rapidly oxidised and thereby brought about the marked improvement noticed in the reaction.

The cultivated soils were generally deficient in exchangeable calcium, probably owing to absorption by crop and loss by drainage. The abnormally large quantity noted in the recently reclaimed lake-area was due to shells of marine molluscs, occurring plentifully, and passing partially into solution on treatment with sodium chloride. The occurrence of appreciable amounts of exchangeable calcium in the different soils suggested that they were less affected by sea-water than the geographical conditions warranted. The cultivated soils were all deficient, and will improve with liming.

As observed by many other workers, there was a general agreement of sign, but no close relationship, between lime requirement and reaction of the soils. All the cultivated soils required lime, but only in moderate quantities. The high requirement of the unreclaimed *Kari* land was probably due to the large amount of free acid. There was a general negative correlation between exchangeable calcium and lime requirement of soils A, B, C and H, but the others, for reasons already stated, indicated the reverse.

WATER-SOLUBLE CONSTITUENTS.

To determine whether occasional inundations with sea-water had appreciably affected the water-soluble constituents of the soils (Chemists' Year Book, 1927, 830), estimations have been recorded as percentages in Table V.

TABLE V.

Soil	Chloride	Sulphate	Calcium oxide	Total solid	Alumina and ferric oxide
A	0·0022–0·0198	0·0016–0·0226	0·0018–0·0070	0·05–0·10	...
B	0·002–0·0154	0·0103–0·078	0·0018–0·0106	0·065–0·5	...
C	0·045	0·103	0·007	0·50	...
D	0·026–0·035	0·034–0·556	0·146–0·280	1·10–1·35	...
E	0·0088	0·0515	0·007	0·10	...
F	0·0617	2·307	0·44	3·375	0·575
G	0·0441	1·0609	0·1526	1·750	0·165
H	0·0133	0·0166	0·0142	0·175	...

The quantities of dissolved salts varied much with locality, but were not abnormal except on the unreclaimed *Kari* soil. Chloride, in particular, was uniformly low, thereby confirming the previous observation that the compositions of the cultivated soils were not affected by occasional inundation with sea-water. The heavy rains also might have washed out the soluble salts from time to time.

BACTERIAL NUMBERS.

To obtain an estimate of bacterial activity in the different soils, platings were made by Thornton's method (*Ann. Appl. Biol.*, 1922, **9**, 241). The average counts, in thousands, were:—A, 440; B, 350; C, 680; D, 355; E, 386; F, only fungi; G, 602; and H, 714. Excessive growth of fungi noticed in all the plates rendered counting difficult.

The very low counts of bacteria indicated that the soil conditions were not favourable to the growth and activity of those highly useful organisms. This was further confirmed by absence of nitrification and, probably, of nitrogen fixation bacteria because of the unfavourable reaction, in all the soils. The early appearance of large numbers of actively growing fungi on the plates indicated, on the other hand, that they (*a*) were favoured by the reaction and the presence of large quantities of straw and sundry vegetation, (*b*) occurred largely in the vegetative condition and (*c*) played an important part in the transformation of organic matter in the different soils of Kuttanad. The foregoing results also showed that, although Thornton's technique gives generally cleaner plates than most other count-media, it fails to keep down ready germination and rapid spread of fungi in some cases.

DISCUSSION.

Although the general physical and physico-chemical characteristics of the soils of Kuttanad were not very different from those of most soils in other parts of the world, yet the natural conditions and the practised systems of agriculture bring them into a class by themselves. Because adequate cultivation and drainage are absent, and large quantities of straw and plant residues rot in them, they (*a*) contained much undecomposed organic matter, (*b*) were generally acid, (*c*) did not contain sufficient aerobic bacteria to bring about useful mineral transformations and (*d*) suffered from a large part of the nutrients being rendered unavailable by profuse vegetation of fungi.

Although rice-straw loses much of its feeding value after soaking under water, it still retains a large amount of cellulosic matter which, if properly utilised, can help to produce a useful synthetic fertiliser (Rege, *Ann. Appl. Biol.*, 1927, **14**, 1). A manure thus prepared will (*a*) be cheap to prepare because it will involve only the collection of waste materials from the soil and will involve no more labour in its preparation than that of occasional stirring, (*b*) become a convenient vehicle for the essential plant nutrients, (*c*) not be leached out by rains or drainage though the different nutrients will be readily available to growing plants, (*d*) contain no fresh decomposable matter and therefore neither turn acid nor encourage growth of undesirable organisms. If dung and cattle refuse, which are generally sold by Kuttanad farmers, be also added to the straw, the value of the manure will be greatly enhanced.

Although scientific advice for the preparation of the manure suited to each locality is to be preferred, a useful beginning can be made with that manufactured by the Agricultural Development Company (E.P., 219, 384), which works on a non-profit basis and therefore ensures the fullest value to the farmer. Adco contains, among other things, useful amounts of calcium, nitrogen and phosphorus, all essential to plant growth. It is quick in its action on rice-straw and converts it into a manure in about two months.

It should be possible to prepare the synthetic manure in the open during the major part of the year, but this may not be satisfactory if exposed to the exceptionally heavy monsoon rains which perhaps will remove the more useful portions. It will be advantageous either to prepare it only during the comparatively dry months or, if prepared during the monsoon, to protect it from being lixiviated.

The manure thus prepared may not supply all the lime required by the more acid soils. A useful scheme will be to apply burnt lime to such soils at the rate of 1–2 tons per acre soon after the summer harvest when the fields are comparatively dry. The liming should be continued until the reaction is corrected.

Although the essential nutrients will be released readily from the synthetic manure, it will be necessary to conduct a few preliminary field trials under the local conditions to determine the precise stage before or during plant growth at which the best results can be obtained.

SUMMARY AND CONCLUSIONS.

1. The natural conditions and the systems of agriculture practised on them place the soils of Kuttanad in a class by themselves, but a study of their physical texture and chemical composition showed that, with exception of the *Kari* lands, they are quite normal.

2. Defects such as acidity, non-availability of essential nutrients and inadequate bacterial activity were traced to accumulation of straw and other plant residues which, owing to difficulties in collection and drying, were generally allowed to decompose in the soil.

3. A scheme for correction of acidity, easy disposal and utilisation of decaying organic matter and improvement of soil fertility has been suggested.

The authors' thanks are due to Dr. N. Kunjan Pillai, Director of Agriculture, Travancore, for his kind interest in the progress of the work and for the numerous facilities placed at their disposal; and to Dr. R. V. Norris, for valuable suggestions and criticism.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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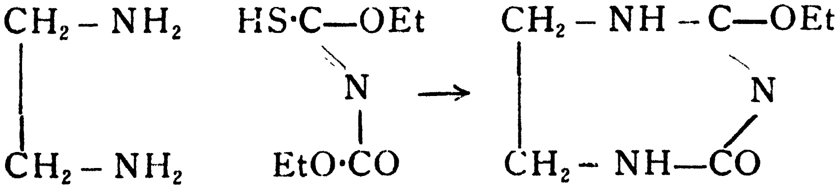
FORMATION OF HETEROCYCLIC COMPOUNDS FROM ETHYL CARBETHOXYTHIOCARBAMATE[1]

By Praphulla Chandra Guha and Shanker Rao A. Saletore.

Ethyl carbethoxythiocarbamate, $CO_2Et{\cdot}NH{\cdot}CS{\cdot}OEt$, was prepared by Delitsch (*J. pr. Chem.*, 1874, **9**, 464) by the action of ammonium sulphocyanide on chlorocarbonic ester in presence of alcohol. It contains two active ester groups—so, it was thought worth while to utilise this compound as a reagent for building up various types of heterocyclic compounds containing nitrogen, by condensing it with different kinds of organic bases, and to compare these results with those obtained by one of us (P.C.G., *J. Indian Chem. Soc.*, 1929, **6**, 65) in investigating the action of organic bases on a similar ester, namely, diethylxanthic formic ester, $CO_2Et{\cdot}CS{\cdot}OEt$. With this object in view, the action of ethylenediamine, *o*-phenylenediamine, hydrazine hydrate, phenylhydrazine, 4-phenyl- and 4-*p*-tolylthiosemicarbazides, di-*p*-tolylaminoguanidine, carbamide, thiocarbamide, aniline, *o*- and *p*-toluidines, *α*- and *β*-naphthylamines and benzidine upon ethyl carbethoxythiocarbamate has now been studied.

A molecule of the thiocarbamate reacting in the *cis*-thiol form with one molecule of ethylenediamine gives rise to 2-ethoxy-7-keto-tetrahydro-1 : 3 : 6-heptatriazine, thus :—



(I)

Together with this heptatriazine, which forms the major portion of the reaction product, there are formed two open-chain compounds, (II) and (III), the formation of which evidently depends on the fact that ethyl carbethoxythiocarbamate reacts with the diamine in the following two different ways :

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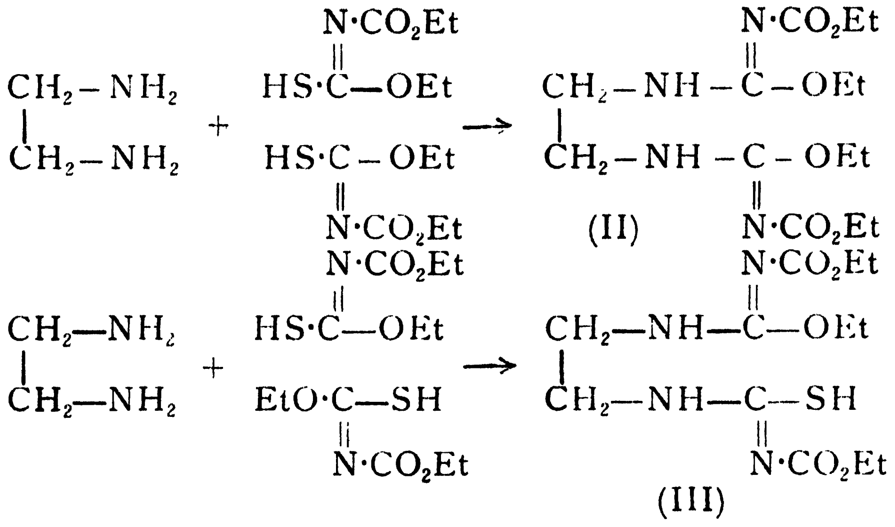
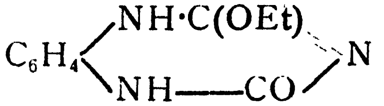
[1] Reprinted from the *Journal of the Indian Chemical Society*, 1929, **6**, 565.

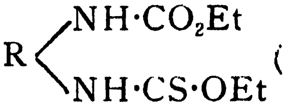


The compound (II) does not contain sulphur and is insoluble in alkali; whereas compound (III) contains sulphur and possesses mercaptanic properties. With *o*-phenylenediamine, the reaction proceeds to yield mainly the sulphur-free heptatriazine compound



(IV), analogous to compound (I).

Diethylxanthic formic ester reacts with ethylene- and *o*-phenylenediamines to yield the corresponding monothiodiurethanes,



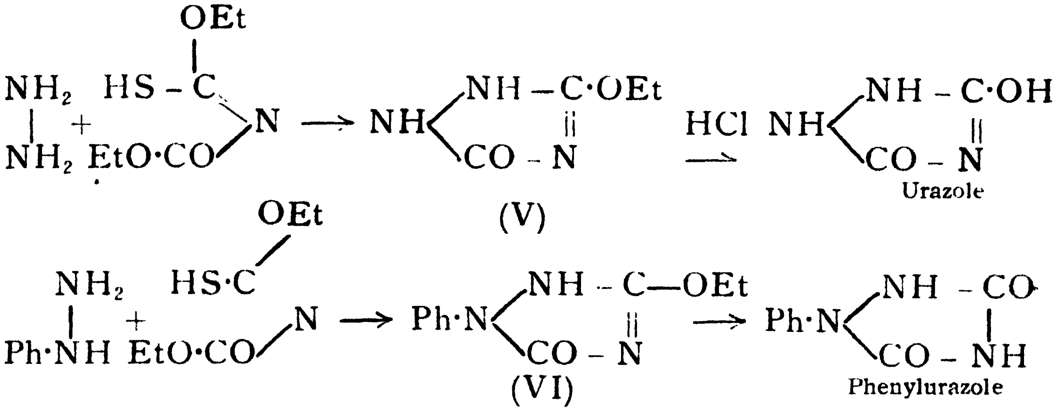
$$R\left\langle\begin{matrix}NH\cdot CO_2Et\\ NH\cdot CS\cdot OEt\end{matrix}\right.$$

(R = phenylene and ethylene), which suffer internal condensation yielding phenylene and ethylene ureas and thioureas (*J. Indian Chem. Soc.*, 1929, **6**, 68).

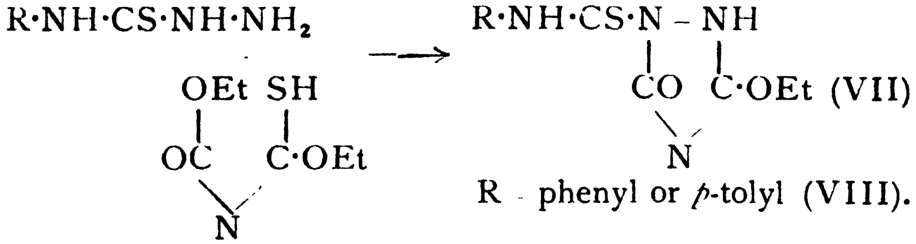
It might be mentioned in this connection that this is not the first occasion on which seven-membered ring compounds from 1 : 2-diamines have been made. Meyer prepared *o*-phenylenemalonamide from *o*-phenylenediamine and malonic acid or ester (*Annalen*, 1903, **327**, 14, 26; 1906, **347**, 34, 45). Thiele and Steimmig prepared a seven-membered compound from *o*-phenylenediamine and acetylacetone (*Ber.*, 1907, **40**, 955). Ethylene and *o*-phenylene biguanides

(Ziegelbauer, *Monatsh.*, 1896, **17**, 648; Dittler, *Monatsh.*, 1908, **29**, 645) are obtained from ethylene- and *o*-phenylenediamines and dicyandiamides. Guha and De (*J. Indian Chem. Soc.*, 1926, **3**, 41) prepared a large number of heptatriazine compounds from *o*-aminophenylhydrazine.

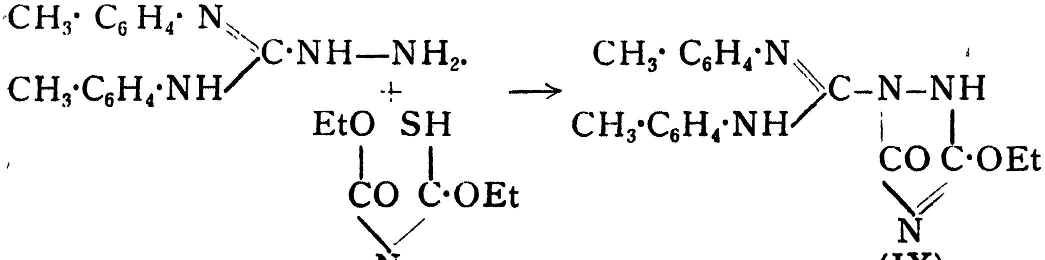
A new method for the preparation of urazole derivatives has been found in the action of ethyl carbethoxythiocarbamate on hydrazine and phenylhydrazine: the reactions evidently proceed thus:



4-Phenyl- and 4-*p*-tolylthiosemicarbazides react with ethyl carbethoxythiocarbamate to yield 1 : 3 : 4-triazole derivatives, which in a sense may be regarded as urazoles.



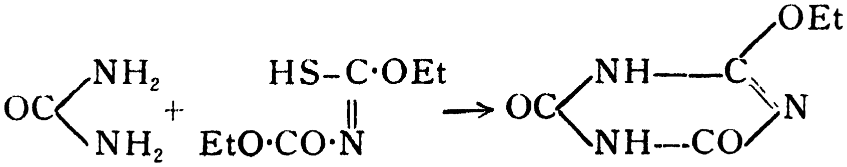
A close examination of the above reaction will reveal the fact that for the formation of these urazole derivatives the hydrazine part of the semicarbazides only takes an active part; to this extent these reactions resemble the formation of urazole derivatives from hydrazine and phenylhydrazine mentioned earlier. An analogous case is to be found in the action of semicarbazide and thiosemicarbazide upon acetoacetic ester as studied by De (*J. Indian Chem. Soc.*, 1926, **3**, 30). The action of ditolylaminoguanidine on the thiocarbamate has also been studied with the result that in this case also the hydrazino-part of the guanidine reacts like that of phenylhydrazine and thiosemicarbazides to yield a urazole derivative, thus :—



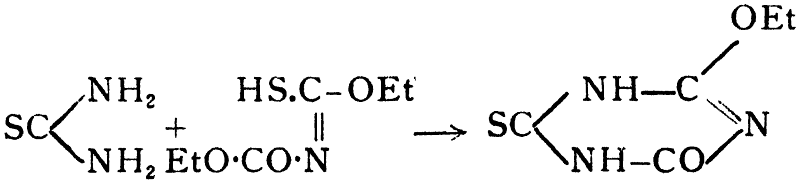
(IX)

Diethylxanthic formic ester, however, reacts with phenylhydrazine and 4-R-thiosemicarbazides to yield the corresponding carboxylic esters Ph·NH·NH·CS·OEt and RNH·CS·NH·NH·CO_2Et, respectively (*ibid*, pp. 66, 72).

Urea and thiourea react with ethyl carbethoxythiocarbamate to yield 6-ethoxy-2 : 4-dioxy-1 : 3 : 5-triazine (X) and the corresponding thio-compound (XI) respectively, thus :



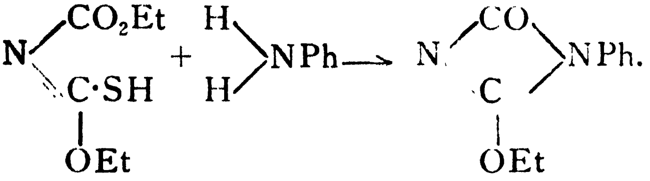
(X)



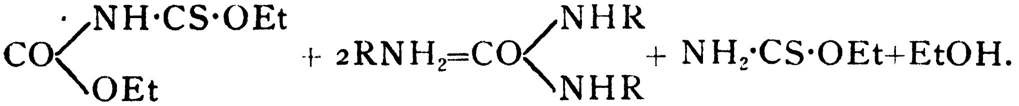
(XI)

Oxy-derivaties of 1 :3 :5-triazines have been synthesised by Pinner (*Ber.*, 1890, **23**, 2919 ; 1892, **25**, 1424), Ephraim (*Bcr.*, 1897, **30**, 2009), and Rapperfort (*Ber.*, 1901, **34**, 1900) by the action of phosgene on aryl amidines. 2 :4-Dioxy-1 : 3 :5-triazines have been prepared by Nencki (*Ber.*, 1876, **9**, 234), Kolb (*J. pr. Chem.*, 1894, **49**, 97), Ostrogorich (*Annalen*, 1895, **288**, 318 ; *Gazzetta*, 1897, **27**, 2, 41) by heating acetylurethane with urea (or acetylurea with urethane). 2 :4 :6-Trioxy-1 :3 :5-triazine (cyanuric acid) and its oxy- and *N*-alkyl derivatives are also known (Meyer and Jacobson, " Lehrbuch der organischen Chemie," Vol. I, Part 2, pp. 1331–1336). 2 :4 : 6-Trimercapto-1 :3 :5-triazine (trithiocyanuric acid) was prepared by Troger and Hornung (*J. pr. Chem.*, 1898, **57**, 357) and by Johnson and Menge (*J. Amer. Chem. Soc.*, 1904, **32**, 362, 370).

By analogy with the formation of ring-compounds as described above, it was expected that ethyl carbethoxythiocarbamate might react with aromatic amines to yield four-membered heterocyclic compounds with alternate carbon and nitrogen atoms according to the following scheme:—



But actually the reaction has been found to proceed in quite a different manner giving rise to *sym*-disubstituted ureas, thus :—

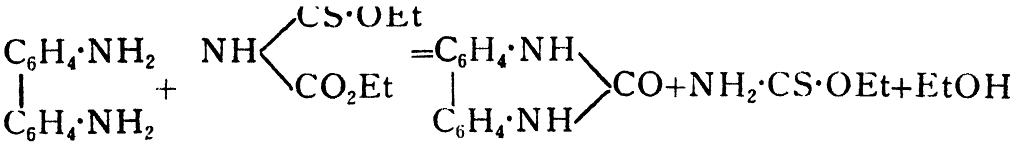


$$\mathrm{CO}\left\langle\begin{array}{l}\mathrm{NH{\cdot}CS{\cdot}OEt}\\ \mathrm{OEt}\end{array}\right. + 2\mathrm{RNH_2} = \mathrm{CO}\left\langle\begin{array}{l}\mathrm{NHR}\\ \mathrm{NHR}\end{array}\right. + \mathrm{NH_2{\cdot}CS{\cdot}OEt} + \mathrm{EtOH}.$$

(XII to XVI)

$$\mathrm{NH_2{\cdot}CS{\cdot}OEt} \xrightarrow{\text{Heat}} \mathrm{Et{\cdot}SH} + \mathrm{HCNO} \text{ (and cyanuric acid).}$$

Benzidine similarly gives carbonylbenzidine, but as it contains two amino-groups in the same molecule, one molecule of the thiocarbamate reacts with one molecule of it, thus :—



$$\begin{array}{l}\mathrm{C_6H_4{\cdot}NH_2}\\ |\\ \mathrm{C_6H_4{\cdot}NH_2}\end{array} + \mathrm{NH}\left\langle\begin{array}{l}\mathrm{CS{\cdot}OEt}\\ \mathrm{CO_2Et}\end{array}\right. = \left.\begin{array}{l}\mathrm{C_6H_4{\cdot}NH}\\ |\\ \mathrm{C_6H_4{\cdot}NH}\end{array}\right\rangle\mathrm{CO} + \mathrm{NH_2{\cdot}CS{\cdot}OEt} + \mathrm{EtOH}$$

Diethylxanthic formic ester reacts with aromatic amines to yield the corresponding thiodicarbomonothiodiarylamides, $\mathrm{NHR{\cdot}CS{\cdot}S{\cdot}CO{\cdot}NHR}$ (Guha and Dutt, *ibid*, p. 65).

EXPERIMENTAL.

Ethyl carbethoxythiocarbamate required for these experiments was conveniently prepared in quantity by the method of Delitsch (*J. pr. Chem.*, 1874, **10**, 118). As described by him, the substance is obtained as pale *yellow* prismatic crystals, m. p. 43° ; whereas, according to Doran (*J. Chem. Soc.*, 1896, **69**, 334) the ester comes out from solutions in rosettes of *yellowish* needles, m. p. 44–45°. We, however, found that repeated crystallisation from light petroleum yielded a pure *white* product which was needle-shaped and melted at 46°. The substance could also be purified more easily by treating the potassium salt with dilute hydrochloric acid in aqueous solution.

The *potassium* derivative was prepared by dissolving the crude ethyl carbethoxythiocarbamate (160 g.) in cold absolute alcohol and adding a concentrated alcoholic solution of caustic potash under ice cooling till there was no more separation of solid. The salt was filtered on the pump, washed with cold alcohol and crystallised from 92 per cent. alcohol; m. p. 225°, yield, 120 g. It is soluble in water and hot alcohol.

Ethyl carbethoxythiocarbamate was isolated from the potassium salt by dissolving the latter in the minimum quantity of water and adding dilute hydrochloric acid. The pale yellow precipitate was crystallised from petroleum (b. p. 60–80°) as white crystalline needles, m. p. 46°, easily soluble in alcohol and ether and also in hot water (Found: N, 7·8; S, 17·89. $C_6H_{11}O_3NS$ requires N, 7·9; S, 18·08 per cent.).

Methyl Derivative.—The potassium salt of the thiocarbamate in alcoholic solution was heated on the water-bath with a slight excess of methyl iodide under reflux for about half an hour when crystals of potassium iodide separated. After filtration, the solution was made free from any excess of methyl iodide by heating on the water-bath, and water was then added when an oily product separated, which solidified on cooling and crystallised from dilute alcohol; m. p. 32–33°. It is extremely soluble in alcohol (Found: N, 7·4. $C_7H_{13}O_3NS$ requires N, 7·32 per cent.).

The *benzyl* derivative was obtained by heating an alcoholic solution of the potassium salt (5 g.) and benzyl chloride (3 g.) for two hours on a water-bath. The precipitated potassium chloride was removed and the filtrate freed from alcohol. The residual oily product was then taken up in ether, washed with water, dried over calcium chloride and the ether distilled. The white solid thus obtained was crystallised from petroleum (b. p. 60–80°) in plates, m. p. 45–46°. It is readily soluble in alcohol (Found: N, 5·3. $C_{13}H_{17}O_3NS$ requires N, 5·24 per cent.).

Reaction with Ethylenediamine: Formation of Compounds (I), (II) *and* (III).—On mixing ethyl carbethoxythiocarbamate (6 g.) with ethylenediamine (3 g.) a vigorous reaction took place with development of much heat and liberation of sulphuretted hydrogen. The hot molten mass solidified on cooling. This was treated with a dilute solution of caustic alkali, when a portion dissolved. The residue was boiled with alcohol (95 per cent.) and filtered. On being cooled, the filtrate deposited a white crystalline product (I) which was recrystallised from alcohol in needles, m. p. 218°. It is soluble in hydrochloric acid from which it is precipitated by alkali; it is sparingly soluble in hot water. It was proved to be free from sulphur (Found: N, 26·8, $C_6H_{11}O_2N_3$ requires N, 26·7 per cent.).

Treatment with Hydrochloric acid.—The compound I (3 g.) was heated on a water-bath with 30–40 c. c. of 6*N*-hydrochloric acid when there was noticed a copious evolution of gas. On cooling, however, only 0·2 g. of a white crystalline solid separated which, after re-crystallisation from dilute alcohol, melted at 225° and was found to be insoluble in acids but soluble in alkalis. It contains 19·5 per cent. of nitrogen which agrees fairly well with the formula of ethylene dicarbamic acid.

Compound (II).—Further concentration of the mother-liquor from the above gave rise to a small quantity of a white crystalline product (m. p. 101–102°) insoluble in alkalis. It crystallises easily from alcohol and does not contain sulphur (Found: N, 15·8. $C_{14}H_{26}O_6N_4$ requires N, 16·1 per cent.). As the amount of the product was small, no further work could be done on it.

Compound (III).—The alkali extract of the reaction mixture, on being treated with dilute hydrochloric acid, precipitated a white flocculent solid, which redissolved on addition of more acid. It was obtained from the acid solution by careful addition of sodium carbonate solution and was crystallised from water in needles, m. p. 123° (Found: C, 44·23; H, 6·92; N, 16·9; S, 9·59. $C_{12}H_{22}O_5N_4S$ requires C, 43·11; H, 6·58; N, 16·8; S, 9·58 per cent.).

2-*Ethoxy*-4 : 5-*benzo*-7-*keto-tetrahydro*-1 : 3 : 6-*heptatriazine* (IV).—Ethyl carbethoxythiocarbamate (6 g.) was condensed with *o*-phenylenediamine (3·6 g.) as described in the previous experiment with this modification, that for completing the reaction, the mixture was heated for about half an hour on the water-bath. But in this case only one product was obtained, which crystallised slowly from dilute acetic acid. It was very difficultly soluble in alcohol, easily in glacial acetic acid and almost insoluble in most other organic solvents. It does not contain sulphur and melts at about 330°; yield, 5 g. (Found: N, 20·48. $C_{10}H_{11}O_2N_3$ requires N, 20·48 per cent.).

3-*Keto*-5-*ethoxy-dihydro*-1 : 2 : 4-*triazole* (V).—Ethyl carbethoxythiocarbamate (3 g.) was mixed with hydrazine hydrate (1 g.) when there was a considerable development of heat and liberation of sulphuretted hydrogen. The product, at first a liquid, partially solidified when it was heated on the water-bath from two to three hours, by which time the evolution of sulphuretted hydrogen ceased. The product was next boiled with alcohol and filtered. The filtrate on cooling yielded a white substance, which was crystallised from the same solvent in needles, m. p. 170–172°. It is moderately soluble in cold water and alcohol and excessively so in hot water. It does not contain sulphur (Found: N, 32·11. $C_4H_7O_2N_3$ requires N, 32·5 per cent.). On boiling for a long time with water or for a short time with hydrochloric acid, the ethoxyurazole is hydrolysed to urazole, m. p. 244°.

3-*Keto*-2-*phenyl*-5-*ethoxy*-*dihydro*-1 : 2 : 4-*triazole* (VI).—When phenylhydrazine (2 g.) was added to ethyl carbethoxythiocarbamate (3·5 g.) there was evolution of sulphuretted hydrogen even in the cold. The mixture was then heated on the water-bath under reflux for nearly two hours, by which time the liberation of sulphuretted hydrogen completely ceased, and a solid product was seen to have been formed. It was cooled and filtered on the pump, washed with a little alcohol and crystallised from the least quantity of alcohol in needles, m.p. 150–151°. Yield, 0·5 g. (Found: N, 20·23. $C_{10}H_{11}O_2N_3$ requires N, 20·48 per cent.). It is very soluble in alcohol and water and does not contain sulphur. When heated with hydrochloric acid for a few minutes, it is converted into Pinner's phenylurazole, m. p. 263°.

3-*Keto*-2-*carbothiophenylamino*-5-*ethoxydihydro*-1 : 2 : 4-*triazole* (VII).—On heating 4-phenylthiosemicarbazide (4 g.) with the thiocarbamate (4 g.) under reflux in an oil-bath at 140°, sulphuretted hydrogen was evolved. After three hours' heating the action subsided. The molten mass solidified on cooling when it was triturated with cold alcohol and crystallised twice from the same solvent in glistening white needles, m. p. 246–248°. Yield, 3 g. (Found: N, 20·92. $C_{11}H_{22}O_2N_4S$ requires N, 21·2 per cent.).

3-*Keto*-2-*carbothio*-p-*tolylamino*-5-*ethoxydihydro*-1 : 2 : 4-*triazole* (VIII).—Ethyl carbethoxythiocarbamate was condensed with 4-*p*-tolylthiosemicarbazide just as in the case of 4-phenylthiosemicarbazide, and a product with similar properties melting at 186–187° was obtained. It was however found to be more soluble in alcohol (Found: N, 20·23. $C_{12}H_{14}O_2N_4S$ requires N, 20·14 per cent.).

2-*Di*-p-*tolylamidino*-3-*keto*-5-*ethoxydihydro*-1 : 2 : 4-*triazole* (IX).—Di-*p*-tolylaminoguanidine (2·5 g.) was heated with the thiocarbamate (1·8 g.) without any solvent for about half an hour when the mixture solidified. The solid after filtration and washing with alcohol was crystallised from the same solvent in rectangular plates, m. p. 229–230°. Yield, 2 g. It is soluble in alcohol and in hydrochloric acid (Found: N, 19·75. $C_{19}H_{21}O_2N_5$ requires N, 19·94 per cent.). On heating with hydrochloric acid for some time, it passed into solution, which yielded on neutralisation with dilute caustic soda solution a solid substance. On crystallisation from alcohol it was obtained in short fine needles, m. p. 256–257° (decomp.).

2-*Ethoxy*-4 : 6-*diketo*-3 : 4 : 5 : 6-*tetrahydro*-1 : 3 : 5-*triazine* (X).—Urea (1·2 g.) and the thiocarbamate (3·5 g.) were heated together when sulphuretted hydrogen was evolved. After 3–4 hours' heating the molten mass became turbid; when cooled in ice and scratched with a glass rod, a solid separated. This was filtered, washed carefully with alcohol and then crystallised from dilute alcohol in minute white needles, m. p. 171–173°. Yield, 0·9 g. (Found : N, 27·63. $C_5H_7O_3N_3$ requires N, 27·4 per cent.). It is soluble in water and alcohol and is free from sulphur.

2-*Ethoxy*-4-*thio*-6-*keto*-3 : 4 : 5 : 6-*tetrahydro*-1 : 3 : 5-*triazine* (XI).—Thiourea (1·5 g.) dissolved in the least quantity of water and a few drops of alcohol was heated with the thiocarbamate (3·5 g.) till sulphuretted hydrogen was no longer liberated. The pasty liquid, on cooling and scratching with a glass rod, gave rise to a small amount of a white solid, which crystallised from alcohol. The product shrinks at 110° and melts at 150°. Repeated crystallisation did not alter the m. p. It is soluble in water and alcohol. Yield, 0·5 g. (Found : N, 24·14. $C_5H_7O_2N_3S$ requires N, 24·2 per cent.).

Reaction with Aniline : *Formation of Diphenylurea* (XII).—Ethyl carbethoxythiocarbamate (3 g.) was heated with aniline (1·5 g.) under reflux for an hour, when there was effervescence with the liberation of a gas smelling of mercaptans. The flask was cooled when white crystals were found to have been formed, which were filtered on the pump, washed with benzene, dried and crystallised from absolute alcohol in white glistening needles, m. p. 235° (Found : N, 13·29. $C_{13}H_{12}ON_2$ requires N, 13·25 per cent.). The substance was proved to be diphenylurea by taking its mixed m. p. with an authentic sample prepared from urea and aniline (Baeyer, *Annalen*, 1864, **131**, 252).

Reaction with p-*Toluidine* : *Formation of Di*-p-*tolylurea* (XIII).—Ethyl carbethoxythiocarbamate was condensed with *p*-toluidine when a white product was obtained; m. p. 270° (Found : C, 74·83; H, 6·3; N, 11·71. $C_{15}H_{16}ON_2$ requires C, 75·0; H, 6·7; N, 11·7 per cent.).

Reaction with o-*Toluidine* (XIV).—o-Toluidine in a like manner gave the corresponding di-*o*-tolylurea as white crystalline needles from alcohol, m. p. 246–247° (Found : N, 11·8. $C_{15}H_{16}ON_2$ requires N, 11·7 per cent.).

Reaction with α- and β-Naphthylamines : *Formation of* (XV) *and* (XVI).—When a mixture of ethyl carbethoxythiocarbamate (2 g.) and α-naphthylamine (3 g.) was heated at 160°, there was effervescence. The heating was continued for an hour during which time the reaction was complete and the molten product was found to have become solid. A small quantity of the adhering tarry product was removed by triturating it several times with benzene and hot alcohol and it was finally crystallised from glacial acetic acid; m. p. 295–296° (Found : N, 8·9. $C_{21}H_{16}ON_2$ requires N, 8·97 per cent.).

Similarly, with β-naphthylamine the corresponding di-β-naphthylurea was obtained crystallising from glacial acetic acid in needles, m. p. 309–310°.

As there seems to be considerable difference in the melting points ascribed to αα′-dinaphthylurea [Delbos, *Annalen*, 1847, **64**, 370; Zinnin, *Annalen*, 1859, **108**, 229; Schiff, *Ber.*, 1879, **12**, 385; Huhn, *Ber.*, 1885, **19**, 2405 (m. p. 270°); Young, *J. Chem. Soc.*, 1897, **71**, 1201 (284–286°); Vittenet, *Bull. Soc. Chim.*, 1874, **21**, 950 (314–315°); Walther and Wldkowski, *J. pr. Chem.*, 1898, **59**, 278 (295–296°)] as also in those of the corresponding ββ′-compound [Huhn (293°); Walther and Wldkowski (300°); Young, *J. pr. Chem.*, 1899, **60**, 256 (289–290°); Vittenet (309–310°); Ekstrand, *Ber.*, 1887, **20**, 1360 (286°)], these two compounds were prepared by two different methods, namely, those of Vittenet and of Young. The products obtained were carefully freed from adhering impurities and were repeatedly crystallised from glacial acetic acid. The α-compound obtained by either method melted at 295–296° and the β-compound at 309–310°. The mixed m. p. of these substances taken with the corresponding substances prepared by our method from the thiocarbamate showed no lowering.

Reaction with Benzidine: Formation of Carbonylbenzidine (XVII). —The thiocarbamate (1 mol.) was heated with benzidine (mol. and a little more) when after a time a solid separated, which was filtered, washed with alcohol and dried. It is very sparingly soluble in alcohol and acetic acid and is insoluble in all other common organic solvents. Hence, the substance could not be crystallised. It becomes brown at 250° and blackens at about 300°. Yield, 3 g. (Found: N, 13·85. $C_{13}H_{10}ON_2$ requires N, 13·33 per cent.). This compound was prepared by Michler and Zimmermann (*Ber.*, 1881, **14**, 2178) by the action of phosgene on benzidine and properties similar to those of our compound were attributed by them to it.

Our thanks are due to Mr. N. C. Dutta, M.Sc., for some preliminary experiments in connection with this work.

Department of Organic Chemistry,
Indian Institute of Science,
Bangalore.

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PETROL-WATER EMULSIONS.

By C. Varadhan and H. E. Watson.

The original object of the present investigation was the preparation of a number of high percentage petrol-water emulsions and their examination as regards suitability for use in internal combustion engines. China (*Proc. Chem. Eng. Group*, 1924, **6A**, 124) states that a 70 per cent. emulsion prepared in a Premier colloid mill with 2 per cent. of ammonium oleate as stabiliser was as efficient as petrol alone when used for a motor-car. After numerous attempts we have been unable to obtain emulsions sufficiently stable for such tests although many different stabilisers were tried; the results, however, have a certain interest in view of their bearing upon the theory of emulsions and consequently the more important of them have been recorded in the present paper.

With all the stabilisers tried it has been possible to obtain emulsions of two types, namely, water in petrol and petrol in water, varying in the time required for separation from a few seconds to years. Measurements have also been made of the interfacial tension between solutions of the stabilisers and petrol, from which it appears that solutions which have the highest interfacial tensions yield stable emulsions containing the highest proportion of petrol. Viscosity determinations show a rapid rise in viscosity with the quantity of petrol.

EXPERIMENTAL.

Experiments were made with the following stabilisers :—Sodium oleate, potassium oleate, ammonium oleate, potassium soap from olive oil fatty acids, ammonium soap from olive oil fatty acids, potassium linoleate, potassium palmitate, ammonium palmitate, potassium stearate, ammonium stearate, gelatin, gum arabic, agar-agar, tallow, dextrin, casein, glycerol, glucose, sucrose, alcohol and rosin. Of these, gelatin, gum arabic, agar-agar, casein and dextrin were found unsuitable as they formed with water thick jellies which could not be emulsified with petrol. Glycerol, glucose, sucrose, alcohol, and rosin were found to have little or no emulsifying power. Consequently, only the remaining substances, the soaps, were investigated in detail.

The petrol used was standard Shell motor-spirit having a density of 0·745. The sodium oleate and ammonium oleate were commercial specimens. The other soaps were prepared from the acids by neutralising with alkali.

The emulsions were prepared as follows :—The stabiliser was dissolved or brought into fine suspension in a measured volume of water, by heat if necessary. The solution or suspension was mixed with a measured volume of petrol and passed many times through a Premier colloid mill (for a description of the mill, see China, *loc. cit.*). The volume of liquid for each charge was usually about 500 c.c. and the gap in the mill between the rotating and the stationary members through which the liquid was passed 0·003 inch. One emulsion was prepared from another by adding to it the requisite quantities of stabiliser, petrol and water and again passing many times through the mill. Preliminary experiments showed that the width of the gap and the number of times of passing through the mill did not affect the properties of the emulsion after about six passages. It was also found that an emulsion had the same properties whether it was prepared directly from petrol and water, or by diluting or concentrating a previous emulsion. The emulsions were milk-white and viscous compared with petrol or water. In some cases they had the consistency of butter. Petrol in water emulsions were usually frothy.

The composition of an emulsion was found by shaking 25 c.c. in a measuring cylinder with an equal volume of 1 : 1 hydrochloric acid and reading the volumes of petrol and water after separation. The type of an emulsion was determined by the drop-dilution method first suggested by Pickering (*J.C.S.*, 1907, **91**, 2002). The indicator method (Robertson, *Kolloid-Z.*, 1910, **7**, 7) was found unsuitable, because in some cases the dye changed the type of the emulsions.

The emulsions were stored in bottles and the time noted at which the formation of a distinct clear layer could be observed. Separation took place in three ways, into (*a*) petrol and water, (*b*) petrol and emulsion, and (*c*) water and emulsion. The first occurred usually with the less stable emulsions of either type, the second with emulsions of water in petrol and the third with those of petrol in water. In the last two cases, separation proceeded slowly for days or even weeks, but after about one month it ceased, and the remaining emulsion was evidently of a very stable type.

The composition of several such stable emulsions was examined when ammonium oleate was used as a stabiliser, and although no exact relation could be deduced, it appeared that there was a tendency for the emulsion to assume a definite composition depending upon the amount of stabiliser present. Thus the 1 per cent. ammonium oleate emulsions mentioned in Table II gave, on separation, emulsions with 28 to 32 per cent. of petrol containing 2·2 per cent. of stabiliser. Two figures for an initial concentration of 2 per cent. stabiliser were 46 and 39 per cent. of petrol with 3·5 and 3·7 per cent. of stabiliser; and one value for the 5 per cent. of stabiliser was 56 per cent. of petrol. Similar results were obtained by Pickering (*loc. cit.*) working with emulsions of a mineral oil in 1 per cent. soap solution. For this concentration he found that the final residual stable emulsion which he calls 'cream' contained about 80 per cent. of oil, irrespective of the amount of oil in the original emulsion.

The approximate hydrogen-ion concentration of each emulsion was found by adding a few drops of B.D.H. universal indicator and noting the colour. P_H for all the emulsions was found to lie between 10·5 and 8·0, but there was no definite relation between its value and the other properties of the emulsion.

Drop-Number Measurements.—As one of the most important factors in the emulsifying action of soaps is the lowering of the interfacial tension between the two liquids to be emulsified, it was considered desirable to make measurements of the interfacial tension between petrol and the soap solutions used for emulsification. For this purpose the 'drop-number' method was employed, absolute values of the interfacial tension not being required, and the values given being sufficiently accurate, particularly as the solutions were all similar.

The drop-pipette (Donnan, *Z. physikal. Chem.*, 1899, **31**, 42; Lewis, *Phil. Mag.*, 1908, **15**, 499) used for the measurements had a capacity of about 5 c.c. and its curved up tip was ground flat. The drops were allowed to rise through the soap solution at intervals of about 5 seconds. The maximum variation recorded between duplicate experiments was 4 per cent. Each determination was repeated several times and the mean value taken as the drop-number. The temperature of the solutions varied from 26° to 29° and was not regulated more closely, as small variations of temperature have only very slight influence on the drop-number (Clayton, *Trans. Faraday Soc.*, 1921, **16**, appendix, 24; *Emulsions and their Technical Treatment*, 36). Three strengths of solution were used in each case, 2, 1 and 0·5 per cent. The drop-numbers could not be found for ammonium palmitate, potassium stearate and ammonium stearate as the solutions formed a thick jelly on cooling. The results are shown in Table I.

TABLE I.

Drop-Numbers of Petrol in Soap Solutions.

Stabiliser	Mean Drop-Number		
	2 per cent.	1 per cent.	0·5 per cent.
Potassium palmitate	390	365	332
Potassium linoleate	342	296	241
Sodium oleate	249	226	201
Potassium oleate	251	215	176
Potassium soap from olive oil ...	210	192	156
Ammonium soap from olive oil ...	90	77	67
Ammonium oleate	Not found	70	60

Drop-Number of Petrol and Water, 38.

The results of some emulsification experiments are shown in Table II. The time for separation is the period which elapsed (in minutes, hours or days) before two distinct layers could be observed. The time for equilibrium to be established was much longer.

TABLE II.

Composition, Type and Stability of Petrol-water Emulsions.

Stabiliser	Stabiliser per cent.	Petrol per cent.	Disperse phase	Time for separation
Sodium oleate	5	73	w.	5 m.
		69	w.	...
		54	p.	2 d.
	2	65	p.	2 d.
		62	p.	3 d.
		53	p.	3 d.
		47	p.	3 h.
		43	p.	2 d.
		42	p.	12 h.
		33	p.	15 m.
Ammonium oleate	5	79	w.	30 m.
		67	w.	6 h.
		60	w.	3 d.
	2	90	w.	5 m.
		69	w.	2 d.
		67	w.	1 d.
		44	w.	25 d.
		27	p.	stable
		26	w.	stable
		15	p.	stable
	1	90	w.	0
		72	w.	1 h.
		69	w.	1 h.
		67	w.	1 d.
		67	w.	5 h.
		44	w.	4 h.
		22	p.	1 d.
Ammonium soap from olive oil fatty acids	4	70	w.	1 h.
		58	w.	stable
		48	w.	stable
		40	w.	stable
		33	p.	stable
		27	p.	stable
		20	p.	stable
	2	70	w.	2 h.
		60	w.	1 d.
		54	w.	2 h.
		40	w.	2 d.
		30	p.	4 d.
Potassium oleate	1	70	w.	10 m.
		70	p.	10 m.
		50	p.	1 d.
		48	p.	1 h.
		44	p.	30 m.
		39	p.	1 h.
		33	p.	15 m.
Potassium linoleate	2	68	w.	0
		64	p.	1 d.
		60	w.	5 m.
		59	p.	1 d.
		50	p.	1 d.
		38	p.	1 h.
		29	p.	1 h.
		20	p.	30 m.
	1	68	w.	0
		58	p.	1 d.
		48	p.	1 d.
		41	p.	1 d.
		30	p.	1 d.
		21	p.	1 d.
		13	p.	1 d.
Potassium soap from olive oil fatty acids	1·8	79	w.	30 m.
		75	w.	5 m.
		74	w.	5 m.
		68	w.	15 m.
		67	w.	15 m.
		64	p.	25 d.
		52	p.	4 d.
		50	p.	1 d.
		41	p.	4 d.
		39	p.	1 h.
		35	p.	3 d.
		29	p.	1 d.
		18	p.	1 d.
		11	p.	1 d.

TABLE II.

(*Composition, Type and Stability of Petrol-water Emulsions.*—contd.)

Stabiliser	Stabiliser per cent.	Petrol per cent.	Disperse phase	Time for separation	Stabiliser	Stabiliser per cent.	Petrol per cent.	Disperse phase	Time for separation
Ammonium palmitate	2	65	w.	30 m.	Potassium palmitate	2	72	w.	10 m.
		62	w.	1 d.			49	w.	2 h.
		49	w.	3 h.			39	w.	2 h.
		40	w.	stable			32	w.	2 h.
		39	w.	1 d.			25	p.	1 d.
		9	p.	0			18	p.	5 m.
	1	67	w.	30 m.		1	69	w.	15 m.
		61	w.	1 h.			61	w.	15 m.
		50	w.	1 h.			53	w.	1 h.
		38	w.	1 d.			52	p.	1 d.
		30	w.	stable			48	p.	1 d.
		18	p.	3 d.			40	w.	1·5 h.
		9	p.	3 d.			30	w.	5 m.
Ammonium stearate	2	68	w.	15 m.	Potassium stearate	2	53	w.	1 h.
		60	w.	stable			38	p.	30 m.
		60	w.	1 d.			30	p.	30 m.
		48	w.	1 d.					
		33	p.	stable		1	70	w.	1 d.
							65	w.	30 m.
	1	67	w.	30 m.			64	w.	2 h.
		59	w.	2 d.			52	w.	1 h.
		49	w.	2 h.			50	p.	1 d.
		40	w.	3 d.			30	p.	stable
		30	p.	5 d.					
		28	p.	stable		0·5	33	w.	1 d.
		16	p.	stable					

All the emulsions mentioned in Table II were so made that the proportion of stabiliser was calculated for the whole emulsion ; thus in a series of emulsions containing say, 2 per cent. of stabiliser, owing to the insolubility of the stabiliser in petrol, the amount dissolved in a given volume of the aqueous phase increased as the proportion of petrol in the emulsion increased. For the sake of comparison, a second series of emulsions was made with petrol and a 5 per cent. solution of sodium oleate so that the proportion of stabiliser in the aqueous phase was constant. The nature of these emulsions is shown in Table III. The symbol W→P indicates that the emulsions were originally of the water in petrol type and changed type on shaking or standing. The figures for stability and viscosity were obtained subsequently to the change.

Viscosity Measurements.—Determinations of viscosity were made for these sodium oleate emulsions both immediately on formation and after various intervals, to determine if possible the changes which took place on standing and to examine the relation between the composition of the emulsions and their viscosity. The results, as might be expected, were irregular, particularly in the case of the less stable emulsions. Measurements were made at 30° in an Ostwald viscometer and it was possible in most cases to obtain concordant values for consecutive readings. On standing, however, many of the emulsions showed signs of separation and had to be shaken before another series of measurements could be made; the viscosity in these circumstances depended to some extent upon the amount of shaking. In spite of this it was possible to obtain average results which showed that, in general, all the emulsions decreased in viscosity on standing, presumably owing to gradual aggregation of the dispersed particles.

Table III gives the viscosities of the different emulsions measured as soon as possible after preparation. Fig. I shows the same values plotted against concentration of petrol and also the figures obtained after the emulsions had stood until the viscosity became approximately constant, usually after about 8 days. Fig. II gives a few of the more regular results obtained for the fall in viscosity. The 52 and 53 per cent. emulsions showed signs of separation after 0·5 and 1·5 hours respectively; consequently all measurements subsequent to the first had to be made after shaking. The two most concentrated petrol in water emulsions containing 69 and 70 per cent. of petrol were fairly stable and extremely viscous. The time of flow for the former was 1,234 seconds falling to 654 seconds in 2 days, during which period no separation had taken place. After 8 days there was separation, and the time of flow after shaking was 352 seconds. The initial time for the second sample was 600 seconds, separation started in 1·5 hours and the time after 26 days was only 218 seconds. The proportional fall was consequently much greater in these cases than it was for less concentrated solutions.

One of the 65 per cent. emulsions gave interesting results being initially of the water in petrol type. The effect of running through the viscometer was to change its type and during the process the viscosity rose to a high value and then fell as shown in the curve marked 65 p.c. in Fig. II. The time of flow then remained approximately constant at 230 seconds for several hours. Next day some separation had taken place and on shaking, the time of flow rose to 480 seconds and remained of this order of magnitude. The portion of the sample not used for viscosity measurements did not change its type until shaken. Two other emulsions were initially of the water in petrol type and exhibited reversal on shaking, but the original emulsions were too unstable to allow their viscosity to be measured.

TABLE III.

Emulsions of Petrol with 5 per cent. Sodium Oleate Solution.

Percentage of petrol	Type	Time for separation	Viscosity (5 p. c. Na—Oleate = 1)
78	W	0	...
72	W	5 m.	(2·30)
70	W	0	...
70	W ⟶ P	30 m.	21·0
69	P	2 d.	43·0
67	W	10 m.	(1·58)
65	W	0	...
65	W ⟶ P	30 m.	17·9
65	W	10 m.	(1·78)
62	P	2 h.	13·0
60	P	1 d.	15·2
60	W	5 m.	...
59	P	1 d.	13·05
55	P	1 d.	19·25
53	W ⟶ P	...	5·22
53	P	1·5 h.	5·44
53	P	1·5 h.	7·32
52	P	30 m.	6·08
52	P	1·5 h.	5·86
50	P	5 m.	...
48	P	1·5 h.	5·35
48	P	1 h.	5·20
45	P	2 h.	5·65
45	P	15 m.	4·32
40	P	5 m.	...
40	P	15 m.	2·04
33	P	0	...
20	P	0	...

DISCUSSION OF RESULTS.

A brief reference to Tables II and III will show that consistent results for the stability of an emulsion are most difficult to obtain, but nevertheless it is possible to form some idea of the relation between composition and stability by examining the general trend of the figures. The emulsions themselves varied from a thin fluid which separated into its constituents in a few seconds to a butter-like mass which was apparently indefinitely stable. In some cases the stability appeared to be accidental, emulsions of the same composition prepared in the same way exhibiting widely different properties. A striking instance of this was observed in the case of two emulsions containing 60 per cent. of petrol with 2 per cent. of ammonium stearate; one of these separated within 24 hours and the other is still stable at the time of writing, about two years later. Similar anomalies have been observed by other workers in an even more accentuated manner probably owing to the fact that most of the emulsions previously studied were produced by shaking, stirring or grinding in a mortar, conditions more difficult to reproduce than those employed by us. For example, Seifrez (*J. Physical Chem.*, 1925, **29**, 839) working with petroleum emulsions seems to have experienced so much difficulty that he remarked, 'It is such idiosyncrasies of emulsions which try the patience of the investigator.'

As already mentioned, emulsions of two types have been obtained with every stabiliser used, the type depending upon the relative quantity of the two constituents. A fairly definite inversion point exists for each stabiliser, emulsions containing more petrol than the quantity corresponding with the inversion point being of the water in petrol type and *vice versa*. In the neighbourhood of the inversion point, emulsions of both types having the same composition could be obtained. There is a marked difference between the ammonium soaps and the other stabilisers, the reason for which is not apparent. With the former the inversion point is in the neighbourhood of 30 per cent. of petrol; for the sodium and potassium salts of unsaturated acids it is at about 65 per cent., for potassium stearate the figure is 50 per cent., and for potassium palmitate 30 per cent. In the last case, however, petrol in water emulsions have been obtained with as much as 52 per cent. of petrol and one emulsion with 0·5 per cent. of potassium stearate was of the water in petrol type although containing only 33 per cent. of petrol. The inversion point did not appear to depend on the concentration of the stabiliser.

In general, the ammonium soaps gave more stable emulsions than the other stabilisers particularly as regards water in petrol emulsions. No emulsion of this type stable for an hour could be produced with the sodium or potassium salts of unsaturated acids, but more stable emulsions were obtained with potassium palmitate and stearate, probably because solutions of these have a tendency to set in a jelly. Except in these two cases the degree of unsaturation of the acid appeared to have little effect on stability.

It is clear from the above that the type of emulsion does not depend on the nature of the stabiliser, but on the relative proportion of the constituents. This is not in agreement with the adsorption film theory of Bancroft (*J. Physical Chem.*, 1913, **17**, 514) according to which a soap with a univalent cation should give only oil in water emulsions. The oriented wedge theory (cf. Finkle, Draper and Hildebrand, *J. Amer. Chem. Soc.*, 1923, **45**, 2780) also indicates that only one type of emulsion should be obtained and thus cannot satisfy the requirements of the present experiments. Clayton (*Emulsions and their Technical Treatment*, 1928, 51) suggests that these general theories may be modified by factors depending on the relative volumes of the solution, so that water in oil emulsions could be obtained with soap stabilisers if the original mixture contained a large excess of oil. This would indicate that the water in petrol emulsions were less stable than those of the reverse type, as found for the sodium and potassium soaps; but it does not account for the ammonium soaps, which in several cases gave equally stable emulsions of both types with the same volume composition, nor does it explain the existence of water in petrol emulsions with potassium palmitate and stearate containing only 30–40 per cent. of petrol.

Within the last few years several authors have described the preparation of emulsions of both types with the same stabiliser, among others Joshi (*Trans. Faraday Soc.*, 1925, **20**, 512) and Seifrez (*loc. cit.*). The former used olive, castor and paraffin oils with soap stabilisers; the latter, petroleum distillates of various densities stabilised with casein. In this case the type of emulsion depended upon the density of the oil. It is thus evident that the simple theories so far put forward are inadequate to explain observed facts and that the nature of an emulsion depends upon a number of complex factors which require a great deal more experimental work for their elucidation than has yet been conducted.

If the results of Table II for the stability of the emulsions be compared with the drop-numbers for the different stabilisers it appears that the most stable emulsions are obtained with the stabilisers which have the lowest drop-numbers, i.e., ammonium oleate and the ammonium soap from olive oil fatty acids. This is the reverse of what might be expected since it is usually considered that emulsification takes place most readily when the interfacial tension is lowest, i.e., when the drop-number is largest. On the other hand it is possible that, once the emulsion is formed, a high interfacial tension tends to prevent aggregation of the globules and thus conduces to stability.

The general relation between the composition of the emulsions and their viscosity has already been mentioned. In the case of petrol in water emulsions the viscosity rises very rapidly with increase in the quantity of disperse phase and it seemed possible that the values might agree with those calculated by Hatschek's equation (*Trans. Faraday Soc.*, 1913, **9**, 80),

$$n = A^{\frac{1}{3}}/(A^{\frac{1}{3}} - 1)$$

where n is the viscosity of the emulsion relative to that of the continuous phase and A is the ratio of the total volume of the emulsion to the volume of the dispersed phase. The calculated values are shown in Fig. I and it will be seen that they are much lower than the experimental figures in the region of high volume concentration to which the equation is considered particularly applicable.

The equation of Arrhenius (*Z. physical Chem.*, 1887, **I**, 285), $\log n = K/A$ with the above notation, contains an arbitrary constant K and hence can be fitted to the experimental values with some semblance of accuracy but an inspection of the curve in Fig. I for which K has the value 0·0186, p being the percentage of petrol and equal to $100/A$, shows that the equation cannot be regarded as representing the true nature of the results.

Over the stable range, the results for the initial viscosities may be represented by the parabolic equation

$$3n = 103 - 4{\cdot}83\, p + 0{\cdot}62\, p^2$$

but this expression has naturally no physical significance. In order to investigate the change in viscosity with concentration and represent the relation between these two factors by an equation, many results more concordant than those obtained in the present experiments are required and these are, unfortunately, extremely difficult to secure.

In conclusion, we wish to express our best thanks to Mr. S. K. Kulkarni Jatkar for assistance throughout the course of this work.

Department of General Chemistry,
Indian Institute of Science,
Bangalore.

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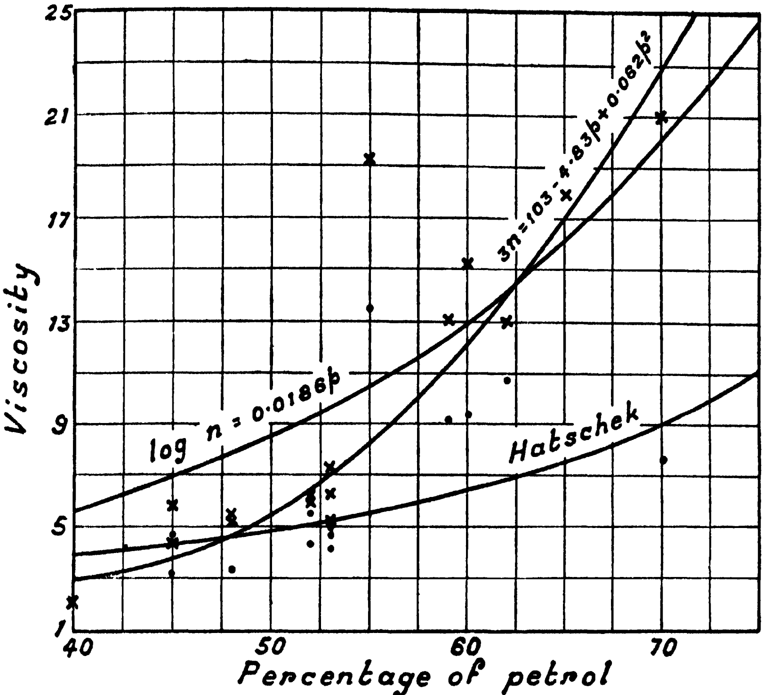
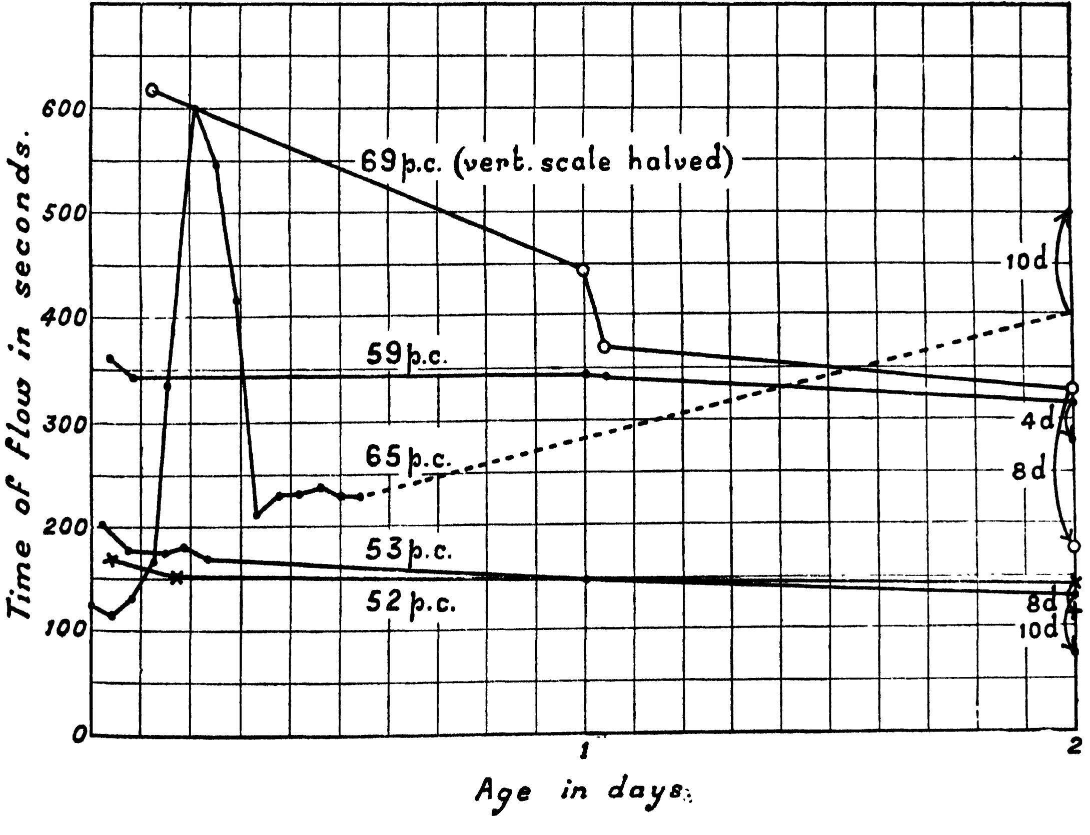
Fig. I



Crosses-initial viscosity : dots-final viscosity

Fig. II



I.—STUDIES ON DEXTRINS. PART I.

Action of amylase from Cholam (*Sorghum vulgare*) on potato starch.

By Vinayak Narayan Patwardhan.

The dextrins form an important group of carbohydrates occurring under various conditions and invariably formed as intermediate products during enzyme-hydrolysis of starch; they possess considerable interest from the academic and the nutritional point of view, but our knowledge of them is comparatively obscure. The only criteria at present available for establishing their identity and purity are reducing power, specific rotation and behaviour on enzyme-hydrolysis. It is therefore not possible to state from our present knowledge whether any of the dextrins so far prepared is a single substance or a mixture.

Musculus (*Compt. rend.*, 1860, **50**, 785) was the first to show that sugar and dextrins were formed simultaneously during the hydrolysis of starch. O'Sullivan (*J.C.S.*, 1872–76), in his systematic studies on the breakdown of starch by diastase showed conclusively that maltose was the sugar formed in the hydrolysis and was always accompanied by dextrins. He stated that the formation of dextrin increased with rise of temperature, more dextrin than maltose being formed between 60° and 70°.

Brown and Heron (*J.C.S.*, 1879, **35**, 596) stated that diastatic hydrolysis of starch takes place by stages, one molecule of maltose being set free at each step accompanied by a lower dextrin. Brown and Morris (*J.C.S.*, 1889, **55**, 449) isolated an amylodextrin by the action of dilute mineral acids on ungelatinised starch in the cold, and showed that it differed from soluble starch. Lintner (*Ber.*, 1893, **26**, 2533) observed that by the action of diastase on starch three dextrins were formed, besides maltose and isomaltose. He named them amylodextrin, $(C_{12}H_{20}O_{10})_{54}$, erythrodextrin, $(C_{12}H_{20}O_{10})_{17}\cdot(C_{12}H_{22}O_{11})$ and achroodextrin, $(C_{12}H_{20}O_{10})_{5}\cdot(C_{12}H_{22}O_{11})$ respectively. Amylodextrin did not reduce Fehling solution, but gave a blue colour with iodine: erythrodextrin reduced Fehling solution and gave a brown colour with iodine, while achroodextrin reduced Fehling solution, but gave no colour with iodine. Ling and Baker (*Proc. C. S.*, 1896–1897, 3) separated two dextrins, maltodextrin-α and maltodextrin-β from the reaction mixture of starch and diastase at 70°. Brown and Millar (*J.C.S.*, 1899, **75**, 315) obtained a 20 per cent. yield of stable dextrin and 80 per cent. of maltose by the action of diastase on starch at 60°.

The dextrin had a reducing power (R), 5·5–5·9 (maltose, 100) and specific rotation, $[\alpha]_D$ 195–196°. When hydrolysed by diastase this dextrin yielded equal quantities of maltose and glucose, the hydrolysis being very slow. Zulkowski and Franz (*J.C.S.*, *Abstr.*, 1896, **i**, 120) showed that dextrins were also formed by heating starch with glycerol at temperatures between 190° and 210°. The action of *Bacillus macerans* on starch (Schardinger, *J.C.S.*, *Abstr.*, 1911, **i**, 181, from *Centr. Bakt.*, 1911, **29**, ii, 188) produced bodies resembling dextrins; some were crystalline and others amorphous, but all were unfermentable by yeast.

Latterly Gatin-Gruzewska (*Compt. rend.*, 1908, **146**, 540, and *ibid*, 1911, **152**, 785) claimed to have separated amylopectin and amylose from the granules of starch. Amylose was soluble in cold water, while amylopectin only gelatinised in hot water. Pringsheim and Eissler (*Ber.*, 1913, **46**, 2959) isolated two kinds of dextrin by the action of Schardinger's bacillus on starch, naming them dextrin-α or tetra-amylose and dextrin-β or hexa-amylose; underlying this nomenclature was the idea that polyamyloses formed the structural basis of the starch molecule. They subjected the polyamyloses to the action of malt diastase, taka-diastase, emulsin, *Penicillium africanum* and yeast. Pringsheim and his collaborators devoted much attention in later years to the chemical constitution of polyamyloses, mention of which will be made in a later paper dealing with that particular aspect.

Ling and Nanji (*J.C.S.*, 1923, **123**, 2666) determined the ratio of amylose to amylopectin in the molecule of starch and found it to be 1 : 2. They found that when alcohol-dried amylase from ungerminated barley was allowed to act on starch-paste at 50°, amylose was converted quantitatively to maltose, while amylopectin was left entirely unaffected. Undried barley amylase, on the other hand, was observed to attack amylopectin and give αβ-hexa-amylose. Ling and Nanji (*J.C.S.*, 1925, **127**, 629) also studied the stable dextrin isolated by Brown and Morris in 1885. The dextrin had the following constants:—R, 14; $[\alpha]_D$ 185°·0 and molecular weight, 1923; it could be hydrolysed by malt diastase, emulsin and maltase to give mixtures of glucose, maltose and isomaltose.

Baker and Hulton (*J.C.S.*, 1929, 1655) observed that precipitated amylase from ungerminated oats hydrolysed potato starch to maltose only, but that when the reaction was stopped before completion, α-amylodextrin (R, 1·5 and $[\alpha]_D$ 184°·1) identical with that obtained by the action of amylase from ungerminated rye on potato starch (Baker and Hulton, *J.C.S.*, 1921, **119**, 805) was the product. Amylase from germinated oats gave a dextrin with R, 9·3 and $[\alpha]_D$ 185°·9, while germinated rye amylase gave a dextrin with the constants R, 10·8 and $[\alpha]_D$ 181°·9. These results of Baker and Hulton do not fit in with the theory first brought forward by Maquenne and Roux (*Compt. rend.*, 1905, **140**, 1303) and later, elaborated by Ling and Nanji (*loc. cit.*), namely, that starch is not a homogeneous substance but is built up of two substances, polymerised amylose and amylopectin.

The work in this laboratory supports the views of Baker and Hulton. During the hydrolysis of potato starch by amylase from cholam (*Sorghum vulgare*) four different dextrins were isolated. To avoid confusion of nomenclature they have been tentatively named Dextrins–I, II, III and IV. Dextrin–I was isolated from the reaction mixture containing cholam malt amylase and potato starch when it just failed to give a purple colour with iodine. Dextrin–II represents the stable dextrin of Brown and Morris. It was obtained when the reaction between cholam malt amylase and potato starch had reached an equilibrium, which was after about 80 per cent. starch had been converted into maltose. Alcohol-dried amylase from ungerminated cholam saccharified starch to the extent of 30 per cent. in sixteen hours ; nearly 66 per cent. of non-saccharine solids could be precipitated from the reaction mixture and were separated into two fractions, Dextrin–III and Dextrin–IV. The former was non-reducing and the latter only feebly reducing.

The crude dextrins were purified by redissolving and reprecipitating from water several times, using 95 per cent. alcohol as the precipitant, till reducing power and specific rotation were found to remain unchanged by subsequent treatment. They were then subjected to prolonged extraction with dry methyl alcohol and later, with 80 to 95 per cent. ethyl alcohol to ensure complete removal of sugar. The dextrins as finally obtained were white amorphous powders soluble in water. Dextrins–I and II dissolved in cold water giving clear solutions, while the other two dissolved only on boiling. The constants for the pure dry substances were as follows :—

Dextrin	I	II	III	IV
Reducing power (glucose 100) ...	3·0	5·0	0	less than 1
Specific rotation, degrees ...	174·0	155·9	170·6	184·2

The constants for Dextrin–IV suggested that it was probably identical with α-amylodextrin of Baker and Hulton.

The dextrins were submitted to hydrolysis by amylases from malted and ungerminated cholam. Dextrin–II was resistant to the action of malted cholam amylase at P_H 7·0, while at P_H 4·67 it was hydrolysed to the extent of about 20 per cent. The other dextrins were rapidly hydrolysed by both the enzymes at the optimal temperature and hydrogen-ion concentration (P_H 4·67). The enzyme from ungerminated cholam attacked all four dextrins more effectively than that from malted cholam and carried the hydrolysis much further. In no case, however, did hydrolysis proceed to completion; under the most favourable circumstances the dextrins were hydrolysed as follows :—

Dextrin	I	II	III	IV
Hydrolysis per cent.	61·6	35·8	66·3	74·8

EXPERIMENTAL.

Preparation and hydrolysis of Dextrin–I.

Malted cholam powder (10 gms.) was extracted with cold water for two hours with frequent shaking, the filtered extract being diluted to 100 c.c. Potato starch (100 gms.) was made into paste with three litres of boiling water and after boiling for a few minutes cooled to 50°, at which temperature the paste and the enzyme were mixed. The reaction was allowed to proceed at the same temperature and stopped by boiling the mixture after it ceased to give a purple colour with iodine. The mixture was then concentrated on the water bath and the dextrin precipitated with 2½–3 volumes of 95 per cent. alcohol. The precipitate was purified in the manner described already.

Hydrolysis of Dextrin–I at 50°.—Flasks containing 50 c.c. of dextrin solution (2 per cent.), 20·85 c.c. of malted cholam enzyme (0·24 per cent.) with 19·15 c.c. of buffer (McIlvaine's and 1 c.c. of toluene were kept in a thermostat. Sugar was determined at intervals.

TABLE I.

Time in hours	Mgms. maltose	
	P_H 4·67	P_H 7·05
1	23·8	...
2	31·2	17·3
4	40·0	...
24	59·6	32·7
48	65·0	37·5

Preparation and hydrolysis of Dextrin–II.

Starch paste and enzyme solution were prepared as before; the reaction proceeded at 45° with 5 c.c. of toluene for 54 hours. The dextrin was obtained and purified as stated above.

Hydrolysis of Dextrin–II at 37° and P_H *4·67.*—Four flasks were arranged, each containing 25 c.c. dextrin solution (2 per cent.) and 15 c.c. of buffer solution with (1) 10 c.c. of malted cholam enzyme (0·2 per cent.), (2) 10 c.c. of ungerminated cholam enzyme (0·2 per cent.), (3) 10 c.c. of mixture of equal volumes of malted and ungerminated cholam enzymes and (4) 10 c.c. of water. The dextrin being very resistant to hydrolysis sugar was determined only after 48 and 90 hours. At the end of the latter period, the percentages of hydrolysis in the different cases were as follows:—(1), 20·8; (2), 35·8; (3), 29·8; (4), 0.

Preparation and hydrolysis of Dextrins–III and IV.

Alcohol-dried amylase (0·5 gm.) was shaken with cold water for half an hour and made up to 100 c.c. after filtration; 100 gms. of potato starch were made into a paste with three litres of water and the paste cooled to 50°. The solutions were mixed and the reaction allowed to proceed at 50° after adding 5 c.c. of toluene. In sixteen hours the paste was observed to be completely liquefied and the coloration with iodine was purple. The mixture was raised to the boiling-point and concentrated on the water-bath to 800 c.c. when 1300 c.c. of 95 per cent. alcohol was added, the precipitate being allowed to settle for 24 hours. The precipitate was observed to be in two layers, a stiff, horny mass below surmounted by a white, fluffy powder; these were separated and dehydrated. On further treatment and purification they were found to be two different dextrins, the horny mass on complete dehydration giving a white powder (Dextrin–III). The yields of crude substances were 35 and 33 gms. respectively; there was not much loss during the purification.

Dextrins–III and IV were only slightly soluble in cold water, but dissolved on boiling, and remained in solution on cooling.

Hydrolysis of Dextrin–III.—Three flasks each containing 50 c.c. of dextrin solution (2 per cent.) and 25 c.c. of buffer solution together with, in flasks 1 and 2, 25 c.c. malted cholam enzyme, and in the third flask 25 c.c. of ungerminated cholam enzyme, were kept in a thermostat at 30°, 0·5 c.c. toluene being added to each flask. Sugar was determined at intervals.

TABLE II.

Time in hours	Mgms. of maltose		
	Cholam Malt		Ungerminated cholam
P_H	7·05	4·67	4·67
24	21·1	31·1	46·6
48	27·6	39·4	56·5
72	31·2	44·5	60·6
92	...	..	70·3

Hydrolysis of Dextrin-IV.—Reaction mixtures were prepared as described in the previous trial except that only two flasks were arranged for malted and ungerminated enzymes at P_H 4·67. The reaction was conducted in a thermostat at 30°.

TABLE III.

Time in hours	Mgms. of maltose	
	Cholam malt	Ungerminated cholam
5	25·6	30·1
22	36·5	55·9
92	54·4	79·0

The composition of these dextrins is unknown, but attempts are being made to determine their nature.

SUMMARY.

1. Four dextrins were isolated from the products of hydrolysis of potato starch, and their reducing power and specific rotation determined; one dextrin appears to be identical with the α-amylodextrin of Baker and Hulton.

2. The dextrins were hydrolysed by the enzymes from malted and ungerminated cholam. The latter attacked the dextrins more readily and carried the hydrolysis farther than the former.

The author has great pleasure in gratefully acknowledging the useful advice and help rendered by Prof. Roland V. Norris during the course of the work.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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II.—AMYLASE FROM RAGI (*ELEUSINE CORACANA*).

By Vinayak Narayan Patwardhan and Nuggehalli Narayana.

The abnormal results obtained as a result of prolonged dialysis of the amylase from *Zea mais* (Patwardhan, *J. Indian Inst. Sci.*, 1929, **12A**, 185) interested the author to extend the investigation to similar enzymes from other seeds. The present investigation deals with the enzyme from ragi.

That the activity of an amylase is diminished after dialysis and that the loss is more pronounced in case of an animal amylase than in one of vegetable origin is well known (Guyenot, *Compt. rend. Soc. Biol.*, 1907, **63**, 768; Brunacci, *Chem. Absts.*, 1910, **4**, 2324; Lisbonne, *Compt. rend. Soc. Biol.*, 1911, **70**, 62; Biery, *Compt. rend. Soc. Biol.*, 1922, **87**, 1111). The activity thus lost by an animal amylase can, however, be restored by the addition of neutral salts and certain amino-acids (Sherman and Walker, *J. Amer. Chem. Soc.*, 1923, **45**, 1960; Haehn, *Biochem. Zeit.*, 1923, **143**, 516). The behaviour of amylases from vegetable sources is not however, so well established. Contradictory evidence is forthcoming regarding loss of activity by barley malt amylase on dialysis, and the possible reactivation of vegetable amylases in general is still an open question (Eadie, *Biochem. J.*, 1926, **20**, 1016). Like the maize amylase, the enzyme from ragi lost its activity on prolonged dialysis and was not reactivated on addition of neutral salts, amino-acids or buffer solutions. The cause of such behaviour might have been the same in both cases, but it was felt that an intensive study of the subject was necessary before any conclusions could be drawn.

The ragi enzyme had greater saccharifying power than cholam malt and maize malt enzymes, but less than that of barley malt amylase. The optimum temperature lay between 55° and 60°. It was less liable to destruction at higher temperatures, which were observed to cause rapid inactivation of the cholam enzyme. The optimum reaction was between P_H 4·86 and 5·07. There was no evidence to show that a thermostable amylase complement occurred with the enzyme.

EXPERIMENTAL.

Malting and preparation of the enzyme.—The methods were identical with those described in the earlier communications (Patwardhan, *loc. cit.*)

Hydrolysis of potato starch by malted ragi amylase.—*(a)* A flask containing 40 c.c. of 2 per cent. starch paste, 30 c.c. water and 2 c.c.

toluene was brought to 30° and 10 c. c. of enzyme solution (0·502 per cent.) added. Sugar was determined at intervals.

TABLE I A.

Time in mins.	Mgms. maltose in 10 c.c.	Colour with iodine
0	0	Deep blue
15	4·8	Deep purple
30	13·4	Purple
60	26·8	Brown
100	38·9	Brown
1430	84·7	Nil

The reaction reached the equilibrium point in 24 hours, when about 80 per cent. of the starch had been saccharified to maltose. Comparative figures for maltose production in 100 minutes from the start of the hydrolysis as observed for different enzymes are given below.

	Amylases Barley	Ragi	Maize	Cholam
Mgms. maltose ...	76·8	38·9	29·0	18·4

(*b*) To 8 c.c. of starch paste (2 per cent.) and 6 c.c. of water at 30° 2 c.c. enzyme solution (0·502 per cent.) was added in a viscosimeter and the readings taken at intervals.

TABLE I B.

Change of Viscosity in terms of time of flow (t) *in secs.*

Time of reading in minutes	1	15	30	45	60	102
t	211·0	125·5	120·5	114·0	114·0	110·0

Effect of change of temperature.

Eight flasks containing 15 c.c. each of starch paste (2 per cent.) were brought to the desired temperature and 15 c.c. of 2 per cent. ragi malt extract added. Readings were taken after hydrolysis for 30 minutes in each case.

TABLE II.

Temperature Centigrade	Mgms. maltose in 10 c.c.	Mgms. ppt. (dry) in 10 c.c.
30	18·9	85·6
40	19·2	83·5
45	22·7	67·9
50	24·2	...
55	35·8	59·3
60	38·2	52·3
65	27·9	77·4
70	25·6	81·5

Effect of Change of hydrogen-ion concentration.

A series of flasks containing 15 c.c. of starch paste and 10 c.c. of McIlvaine's buffer solution were brought to 30° and 5 c.c. ragi malt extract (6 per cent.) added to each. Readings were taken after hydrolysis for 30 minutes.

TABLE III.

P_H	Mgms. maltose in 10 c.c.	Mgms. dry ppt. in 10 c.c.
7·0	19·5	175·3
6·6	27·4	190·5
6·15	28·5	175·0
5·96	28·5	173·0
5·8	29·4	171·4
5·6	29·4	163·8
5·26	31·7	164·8
5·07	32·3	159·4
4·86	32·3	155·2
4·67	29·9	163·0
4·50	29·0	160·5
4·34	29·0	164·4

Effect of dialysis on ragi malt enzyme.

Tubes prepared from 6 per cent. collodion were used for dialysis. All dialyses were carried out in a cold chamber between 0° and 3° with toluenated water in presence of toluene.

(*a*) 0·502 per cent. enzyme when dialysed lost its activity as shown below:—

No. of days ...	0	2	5	9	9
c.c. $KMnO_4$ for 10 c.c.	4·70	3·50	3·05	1·05	1·10 (Salt added to the last one)

(*b*) Ragi malt extract (22·5 per cent.) lost its activity practically completely in sixteen days. Extract in a glass tube kept in the same dialyser as control had lost only a little of its activity even after 28 days.

No. of days	0	3	16	17	28
c.c. $KMnO_4$ required for 10 c.c. (corrected) ..	9·30	5·30	0·05	0·05	...
Control—$KMnO_4$ required for 10 c.c. (corrected)	9·30	...	..	...	8·00

The activity could not be regenerated by addition of salt, buffer solutions of known P_H or asparagine. The details of the trials were the same as those described in the paper on maize amylase.

(*c*) Experiment to test the regenerating action of boiled enzyme.

Three flasks, A, B and C each containing 12·5 c.c. of dialysed enzyme and 12·5 c.c. of 2 per cent. starch paste were treated as follows:—To A, 7·5 c.c. of boiled, undialysed enzyme was added, to B, 7·5 c.c. of undialysed, unboiled enzyme, and to C, 7.5 c.c. of distilled water. The volumes of permanganate required after 1 hour at 30° were, A, 0·05; B, 4·85; and C, 0·40 c.c. respectively. The results show clearly that the boiled and dialysed enzymes were inactive, singly and together.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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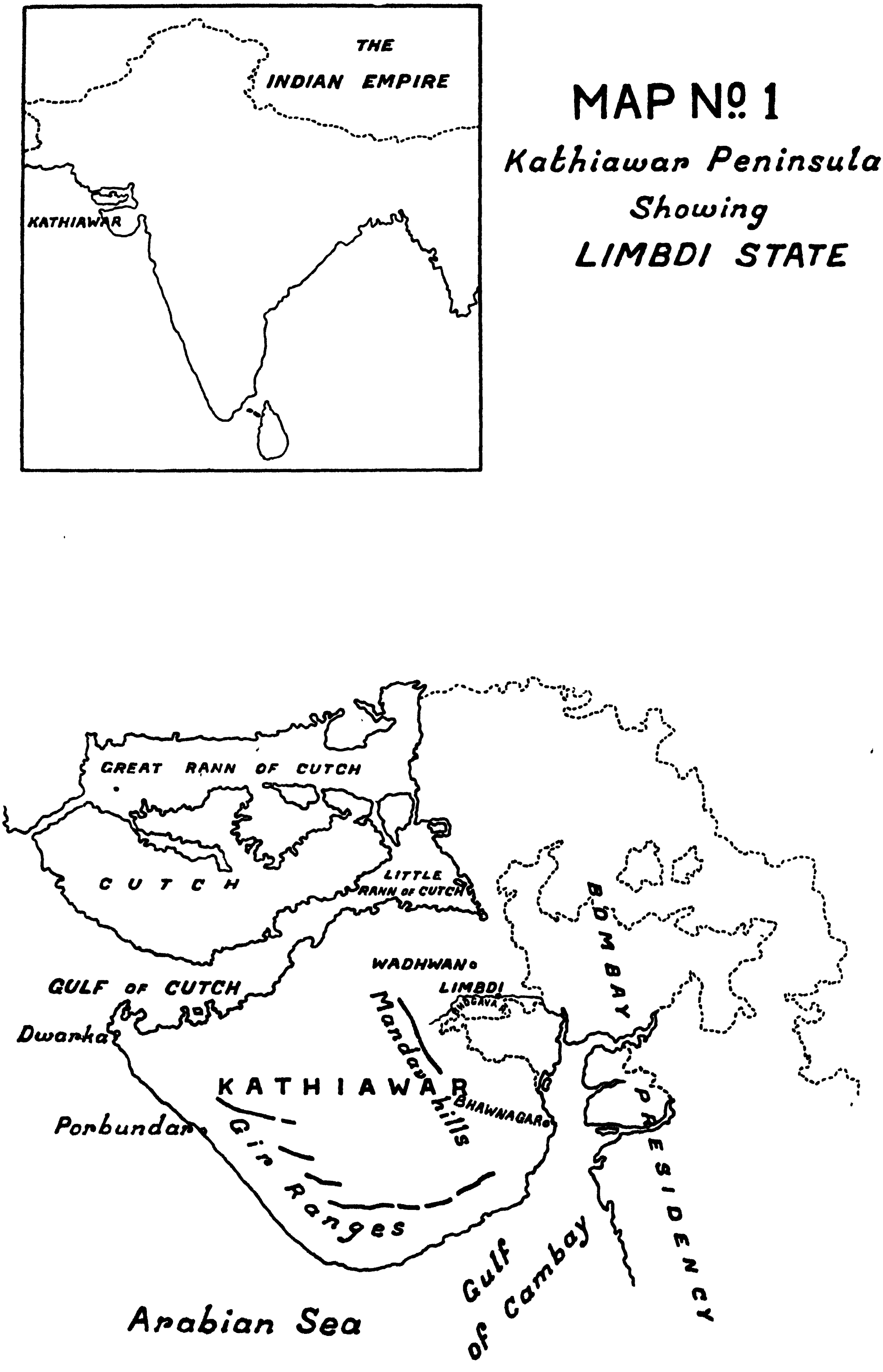
MAP Nº 1
Kathiawar Peninsula
Showing
LIMBDI STATE



SOIL SURVEY OF THE NALKANTHA DISTRICT (LIMBDI STATE) AND ITS SIGNIFICANCE.

By C. V. Ramaswami Ayyar.

Limbdi is one of the native states in the Kathiawar Peninsula and is situated between 22°30′ and 22°37′ N. Lat. and 71°44′ and 71°52′ E. Long. It covers an area of about 350 sq. miles and has been divided into several districts of which Nalkantha is the largest and occupies about 100 sq. miles. The surface of the Kathiawar peninsula is for the most part undulating with low ranges of hills running in different directions (Map 1). The physical features suggest that it once might have been an island or a group of islands of volcanic origin, the upheaval of 1820 (*Imp. Gaz. India*, 1908, **15**, 170) having led to its organic connection with the continent. Along the south of the peninsula the Gir ranges of hills run closely parallel to the coast. The Mandev hills situated in the far west of the Limbdi State and at right angles to the Gir ranges may be described as outcrops of the latter. Most of the intervening landscape is quite even and completely free from vegetation.

During the severe south-west monsoon the Gir and Mandev hills act as barriers to the rain-bearing clouds and bring about a fairly heavy rainfall of 50 to 60 inches in the coastal regions and in some parts of the interior; but only exhausted clouds pass over the Mandev hills to Limbdi resulting in 20 to 25 inches of rain. This forms the total annual rainfall of the State since no rain is received during the rest of the year, and even this often falls in torrential showers during a very short period. Although the wet months are from June to September there are hardly 25 rainy days, the rest of the year being characterised by bright sunny days till the following monsoon.

The soils of Kathiawar are basaltic, showing only slight variations in their texture and general behaviour and possessing high retentive power for moisture. The sunny weather following the monsoon may be expected to assist considerably in the cropping of the tract.

Limbdi being one of the states situated in the peninsula is largely influenced by these climatic factors and especially so the Nalkantha district, for it is the most low-lying part of the State. This district forming the easternmost part of Limbdi is a belt of salt lands having a long lagoon, the Nal, which formed until recent times a connecting link during the rains between the Gulf of Cambay and the Little Rann of Cutch. The lagoon occupies over 40 sq. miles serving as a reservoir for the drainage from higher regions.

The general slope of the district is from west to east, i.e., from Wadhwan to the Gulf of Cambay and also from north-west to south-east; the fall in level being 1 in 1000 the country is quite even. The river and drainage systems are few, most of them being in flood during the monsoon with the exception of Brahman river which remains a live stream throughout the year (Map 2). Apart from these water-sources there are numerous tanks scattered over the district serving as rain-water reservoirs to be used for cultivation and drinking purposes.

After the upheaval of the peninsula, this low-lying tract was rendered fit for cultivation by the constant deposition of silt over a very long period. Even now, salty sub-strata at a depth of 3 to 4 feet along with salty subsoil water are found below the silt. Because of the silting in this tract the soils under study are not of the same nature as in the rest of the peninsula; the conditions facilitating cultivation both in the monsoon and the post-monsoon periods make the moisture available for the crops raised.

The soils have been arbitrarily grouped in three classes according to the type of crops grown, and the purposes of revenue assessment are thus well served. The soil and subsoil show considerable variations. The first two classes are mostly black clayey soils with retentive power for moisture, but without good drainable subsoil. The third class soils are sandy with good drainage but of low fertility. There is yet another type, the Khara lands, which are alkaline and are being gradually reclaimed for cultivation by systematic bunding to assist the deposition of silt.

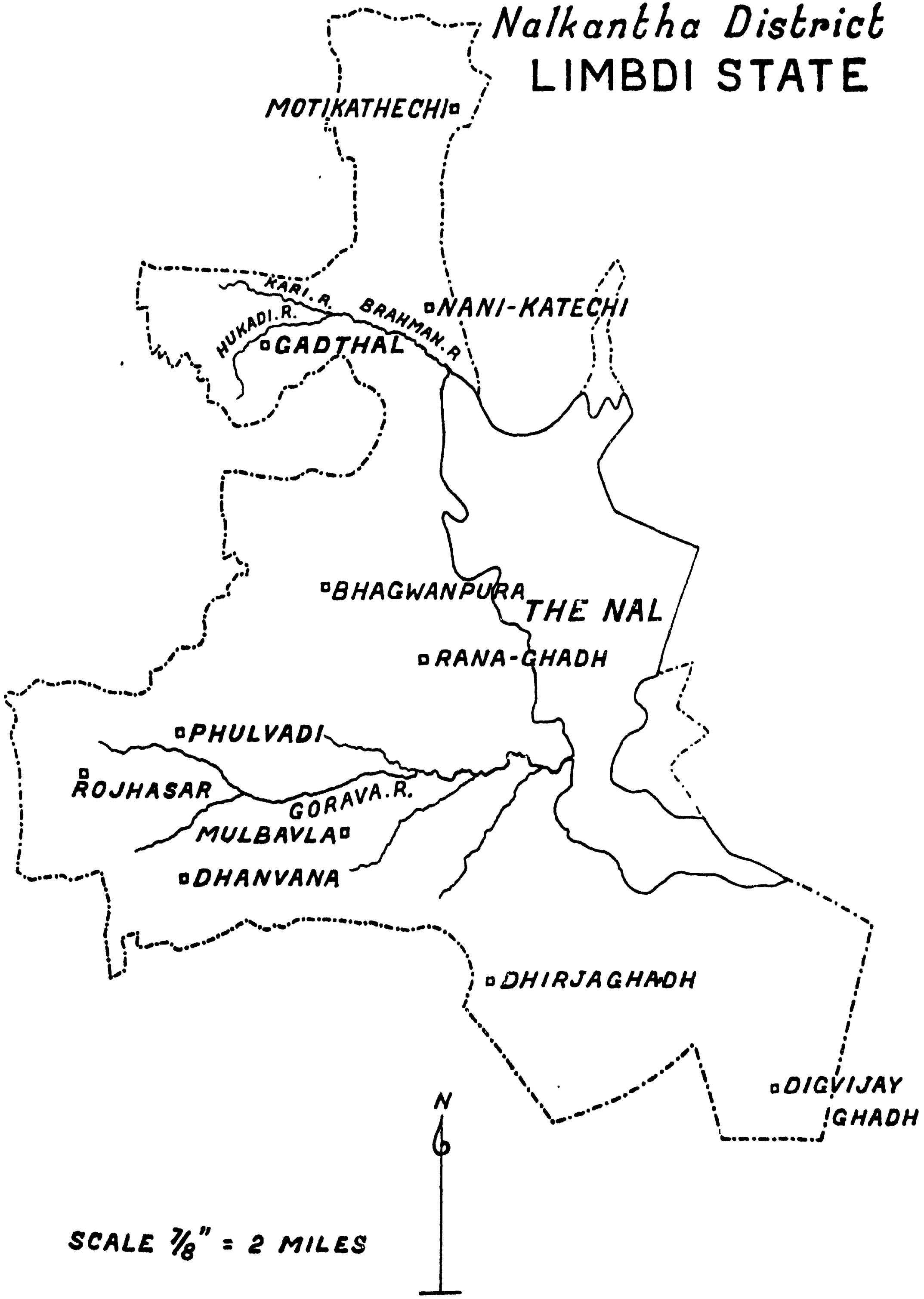
A study of the general character of soils in the district together with observations on the stand of crops suggests that the area is of low fertility. The farmers have no proper understanding of the principles of cultivation, manuring and cropping which in addition to the poverty of soils leads to low productivity.

The soil survey of the Nalkantha district described in this communication comprises the most important aspect of an extended

MAP No. 2

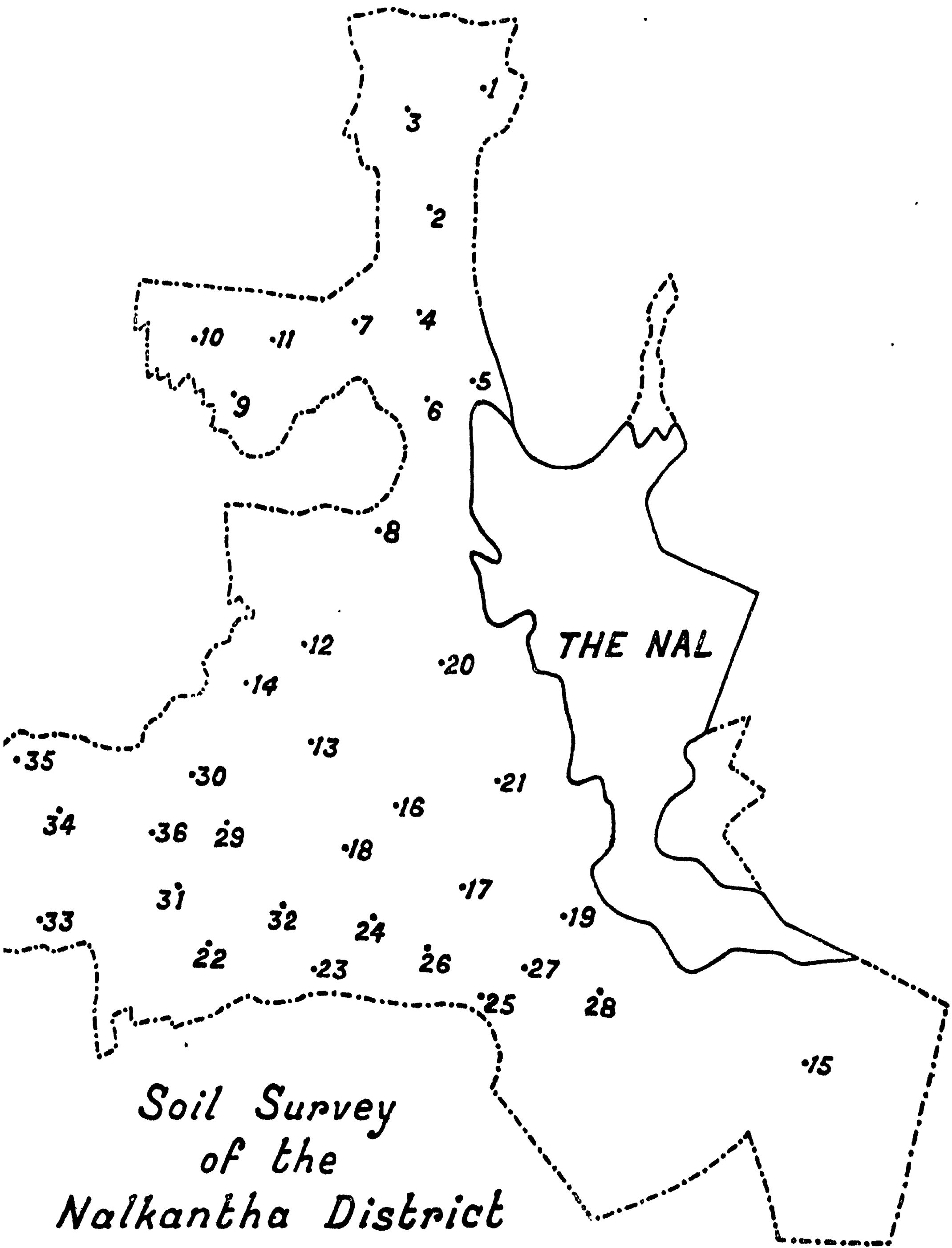
Showing the Villages in Nalkantha District

LIMBDI STATE



MAP No. 3

Showing position of the soil samples



Soil Survey
of the
Nalkantha District
LIMBDI STATE

investigation into the present agricultural condition of the tract. The enquiry formed a guide to a general recommendation for the amelioration of the agricultural prosperity of the colony; based on these results a scientific programme for stimulating production can be inaugurated side by side with an improvement in the existing state of agriculture. A study of soils was undertaken at the invitation of the Limbdi Durbar and a detailed report was presented embodying a number of recommendations concerning the practice of agriculture. The present paper deals with a study of the soils.

COLLECTION OF SOIL SAMPLES.

In March 1928, the author made an extensive tour in the area under discussion and selected fields in each class, collecting representative samples for examination in the laboratory. The district was divided into blocks of four square miles in extent, samples of soil being drawn from each block. Care was taken to include soils from each class, those pertaining to a particular class being treated as an individual sample. Pits 4 feet square and 4 feet deep were dug in all cases, and a representative sample taken by mixing samples obtained from three fields under each class within a specified area, the top nine inches of soil being chosen for the study.

Pits were dug for the study of the soil *in situ*. This profile study furnished full details regarding the character of the soil, subsoil, disposition of the moist layer from the surface, location of the water table and similar information that could be gathered on the field for guidance in the interpretation of the laboratory results. In addition to the above, a thorough enquiry into the cropping history of the respective fields with special reference to cultivation methods, manuring, the general systems of cropping including the out-turn from crops raised were also recorded. From the tract in question 36 samples of cultivated soil and 20 samples of Khara soils were collected for this study. In Map 2 the general configuration, the river and drainage systems and the several villages in the district are shown. Map 3 gives the position of soil samples collected from the different villages. The samples were analysed to determine their chemical and mechanical composition with a view to finding their manurial requirements and their behaviour in cropping. The chemical analysis comprised the estimation of total and available phosphoric acid and potash, nitrogen, lime and magnesia; whereas the mechanical analysis involved the estimation of the proportion of different grades of particles in order to understand their moisture relationship with reference to the peculiar meteorological conditions of the tract. The results of chemical analysis are given in Table I.

TABLE I.

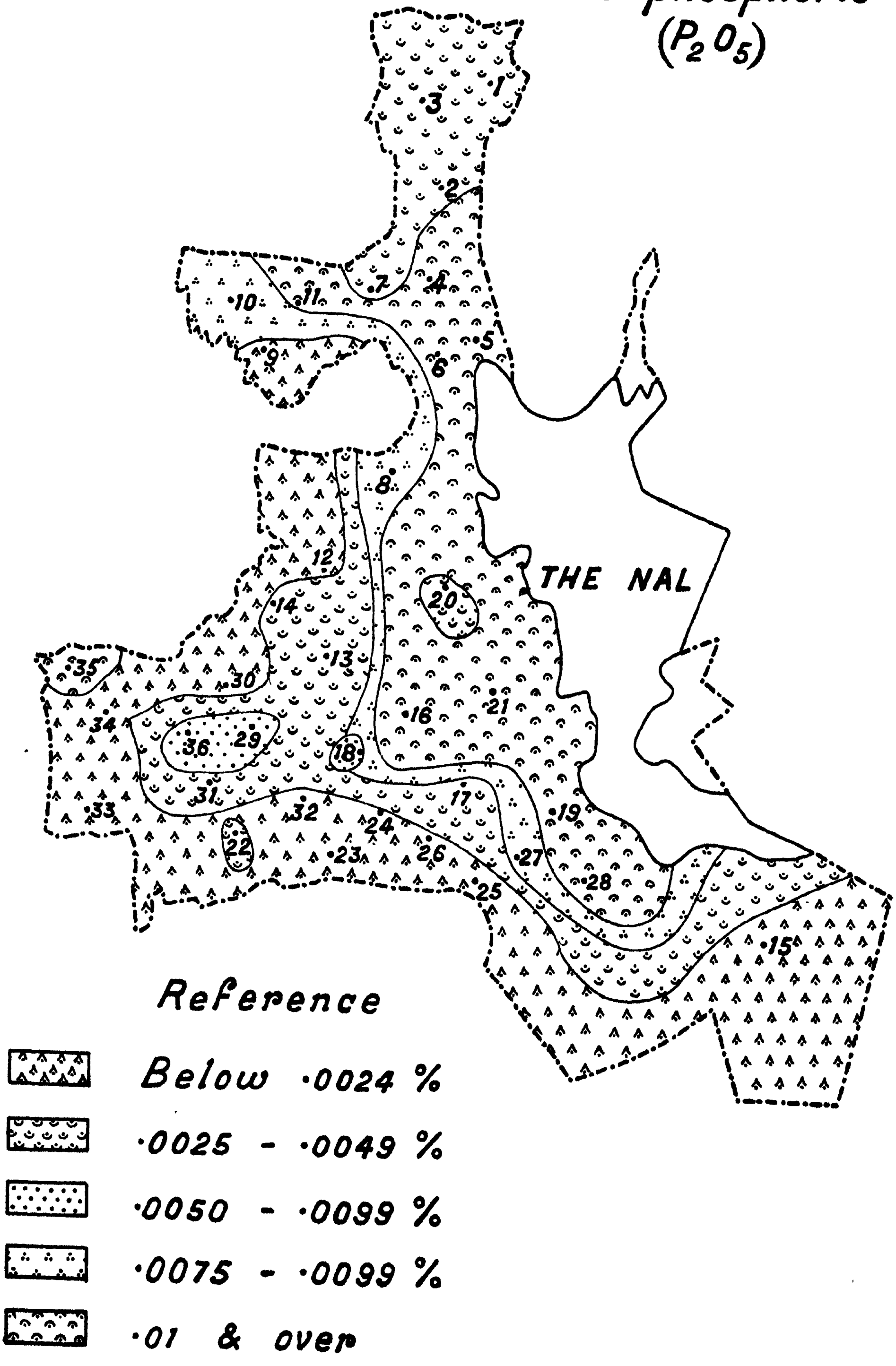
Chemical Composition of Soils.

Nalkantha District, Limbdi State.

Class of soil	Lab. No.	Village	Nitrogen per cent.	Lime per cent.	Magnesia per cent.	Ratio MgO : CaO.	Phosphoric acid, P_2O_5 per cent.		Potash, K_2O per cent.	
							Total	Available	Total	Available
FIRST CLASS	1	Mothikathechi	0·034	2·72	0·82	1/3·32	0·033	0.0034	0·50	0·0197
	4	Nanikathechi	0·023	0·75	0·41	1/1·83	0·048	0·0244	0·37	0·0234
	8	Gadthal	0·027	3·78	1·50	1/2·52	0·041	0·0081	0·47	0·0206
	12	Bhagwanpura	0·029	7·82	1·03	1/7·60	0·041	0·0009	0·49	0·0415
	15	Dhigvijayagadh	0·050	6·22	1·04	1/6·00	0 114	0·0009	1·30	0·0347
	16	Ranagadh	0·038	2·85	0·86	1/3·31	0·061	0·0203	0·56	0·0456
	22	Dhanvana	0·020	3·76	1·58	1/2·40	0·046	0·0036	0·31	0·0156
	25	Mulbavla	0·025	6·87	0·84	1/8·18	0·050	0·0009	0·36	0·0360
	29	Phulvadi	0·034	0·92	0·92	1/1·00	0·024	0·0051	0·20	0·0082
	33	Rojhasar	0·045	9·68	1·39	1/7·00	0·044	0·0009	0·33	0·0109
SECOND CLASS	2	Mothikathechi	0·032	2·04	0·65	1/3·14	0·013	0·0038	0·39	0·0180
	5	Nanikathechi	0·036	0·50	0·41	1/1·20	0·034	0·0161	0·42	0·0187
	9	Gadthal	0·028	9·02	1·26	1/7·16	0·040	0·0009	0 39	0·0286
	13	Bhagwanpura	0·034	2·94	1·65	1/1·77	0.032	0·0047	0·42	0·0156
	17	Ranagadh	0·043	2·25	0·59	1/3·81	0·036	0·0042	0·44	0·0143
	23	Dhanvana	0·022	3·51	0·83	1/4·20	0·030	0·0012	0·32	0·0238
	26	Mulbavla	0·034	4·91	0·53	1/9·23	0·051	0·0009	0·44	0·0442
	30	Phulvadi	0·034	5·32	1·15	1/4·63	0·045	0·0014	0·37	0·0415
	34	Rojhasar	0·031	6·91	1·85	1/3·73	0·046	0·0009	0·44	0·0245
THIRD CLASS	3	Mothekathechi	0·033	6·47	0·55	1/11·80	0·046	0·0036	0·25	0·0199
	6	Nanikathechi	0·028	2·45	0·44	1/5·57	0·031	0·0108	0·40	0·0145
	10	Gadthal	0·055	3·16	0·63	1/5·00	0·053	0·0082	0·53	0·0190
	14	Bhagwanpura	0·028	2·82	0·61	1/4·62	0·025	0·0032	0·26	0·0156
	18	Ranagadh	0·032	3·74	0·56	1/6·56	0·026	0·0065	0·23	0·0163
	24	Dhanvana	0·024	6·29	0·54	1/11·50	0·058	0·0009	0·30	0·0129
	27	Mulbavla	0·046	1·34	1·79	1/0·75	0·047	0·0099	0·29	0·0204
	31	Phulvadi	0·039	2·95	0·57	1/5·17	0·039	0·0026	0·36	0·0578
	36	Rojhasar	0·035	1·23	1·33	1/0·92	0·032	0·0057	0·27	0·0721
FOURTH CLASS	7	Nanikathechi	0·027	0·40	0·42	1/0·95	0·011	0·0030	0·38	0·0179
	11	Gadthal	0·036	1·67	0·79	1/2·11	0·037	0·0109	0·46	0·0190
	28	Mulbavla	0·034	0· 72	0·51	1/1·41	0·025	0·0125	0·21	0·0122
	32	Phulvadi	0·029	8· 34	0·64	1/13·00	0·035	0·0014	0·19	0·0122
		RANAGADH								
	19	Paddy Soil Komad	0·048	1·46	1·57	1/0·93	0·047	0·0106	0·51	0·0612
	20	,, Wari	0·035	0·94	0·76	1/1·23	0·022	0·0044	0·43	0·0687
	21	,, Experimental	0·074	1·56	1·21	1/1·21	0·060	0·0361	0·47	0·0564
		ROJHASAR								
	35	2nd Class Special	0·025	0·61	0·68	1/0·90	0·031	0·0152	0·24	0·0224

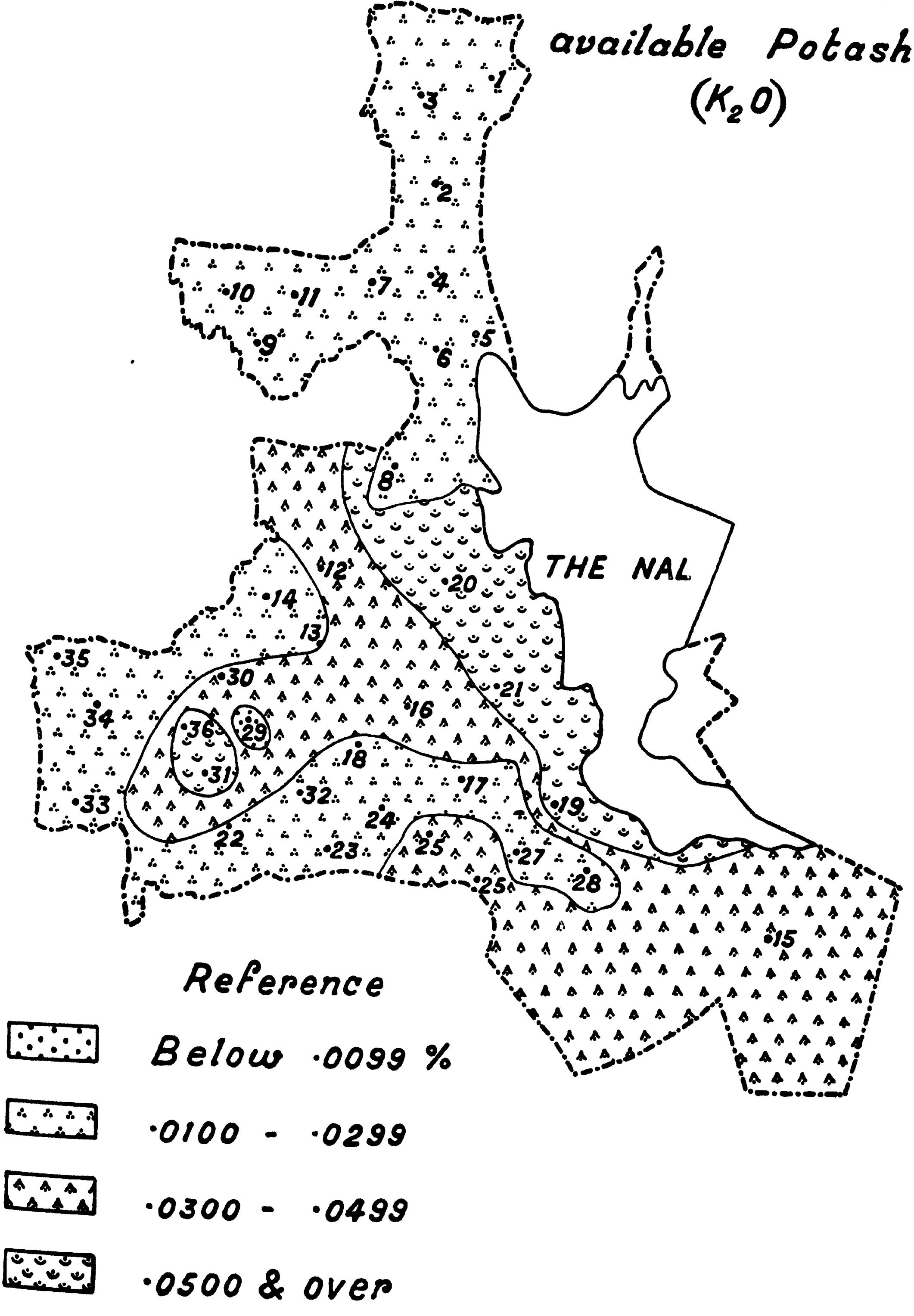
MAP No 4

Distribution of available phosphoric Acid (P_2O_5)



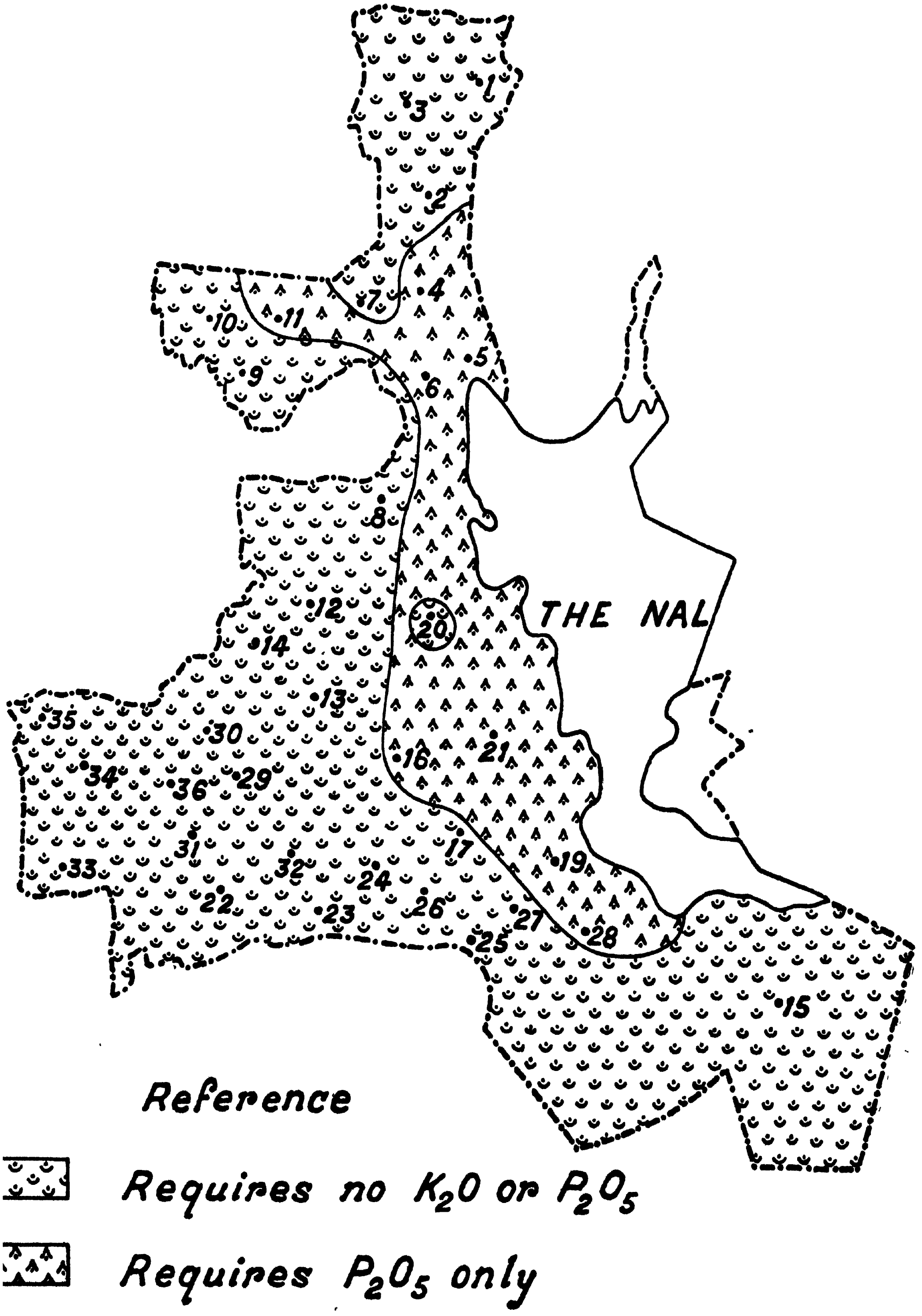
MAP No. 5

Distribution of available Potash (K_2O)



MAP №6

Manurial Requirements



Available plant nutrients were estimated by Dyer's citric acid method as modified by Hall and Ames (*J. Chem. Soc.*, 1906, **89**, 205). Soils containing less than 0·01 per cent. of available phosphoric acid were considered likely to respond to the application of phosphatic manures. The corresponding value for potash was fixed at 0·005 per cent. Soils having values lower than 0·005 per cent. were looked upon as requiring potassic manures.

The distribution of the several manurial ingredients have been plotted in Maps 4 to 12.

DISTRIBUTION OF AVAILABLE PHOSPHORIC ACID.

Map 4 shows the distribution of available phosphoric acid throughout the district contoured between certain limits. It is evident that the only soils which contain over 0·01 per cent. available phosphoric acid and which consequently are not likely to respond to phosphatic manures are those in the proximity of the Brahman river and the tract of land lying within a mile to the west of the Nal. Another small isolated patch rich in available phosphoric acid is in the far south-western corner of the district. With these exceptions the whole tract is highly deficient in phosphates.

DISTRIBUTION OF AVAILABLE POTASH.

Map 5 shows the distribution of available potash. The results judged on the standard fixed show a fair supply of this ingredient indicating that application of potassic manures is not necessary; they confirm the observation that the generality of Indian soils are well supplied with potash, deficiency in this being very rarely encountered.

Map 6 indicates the immediate manurial requirements of the soils. It will be evident that only 20 per cent. of the district contains sufficient available phosphoric acid and potash. Over 80 per cent. of the soils require only phosphate.

It is interesting to observe that the area which is not in need of either phosphoric acid or potash is located within a mile from the Nal, extending from north-west to south-east. This part of the district was till recently a part of the Nal and impregnated with salts; but in the course of a few decades the lands have been reclaimed and the harmful salts removed by frequent application of water. With the removal of salts the dormant plant nutrients became available to the cultivated crops which consequently gave a higher out-turn. Hence a higher amount of plant-food is indicated, and is supported by the available phosphoric acid and potash figures. Barnes and Barkatali

(*Agri. J. India*, 1917, **12**, 379) observed great acceleration in the process of nitrification and the biological processes in general on all the reclaimed lands, lending further support to the foregoing conclusions.

DISTRIBUTION OF TOTAL PHOSPHORIC ACID AND POTASH.

The immediate crop requirements can only be met from the supply of available plant-foods, whereas knowledge of the absolute amounts of phosphoric acid and potash affords a measure of the reserve of these materials which under favourable conditions may gradually become available as plant-food. This is especially so under cultural conditions where manuring is not practised, as in this tract; the amount of total plant-food and the rate at which it is rendered available are two important factors in estimating the comparative fertility of different soils.

The distribution of total phosphoric acid and potash (Maps 7 and 8) shows that these soils contain on an average nearly ten times as much potash as phosphoric acid. This fact points distinctly to the necessity of supplying phosphoric acid in preference to potash. Moreover, the total amount of phosphoric acid in these soils is not sufficient to maintain a normal supply of available phosphoric acid to the crops. Only 15 per cent. of the soils are fairly well supplied with enough reserve phosphate, the rest being comparatively very poor. Unless augmented by a liberal application of phosphatic manures, this reserve is certain to be depleted in continuous cropping, leading to much poorer out-turn from crops than at present.

Norris (*Memoirs Dept. of Agr. in India, Chem. Ser.*, 1923, **6**, 254) emphasised the importance of increasing the reserve phosphate in soils, having observed a rapid depletion of this constituent from soils which, when supplied, exerted beneficial influence on crop-yield ; hence he considered phosphoric acid as a limiting factor in crop production. This observation lends further support for augmenting the reserve phosphoric acid in soils.

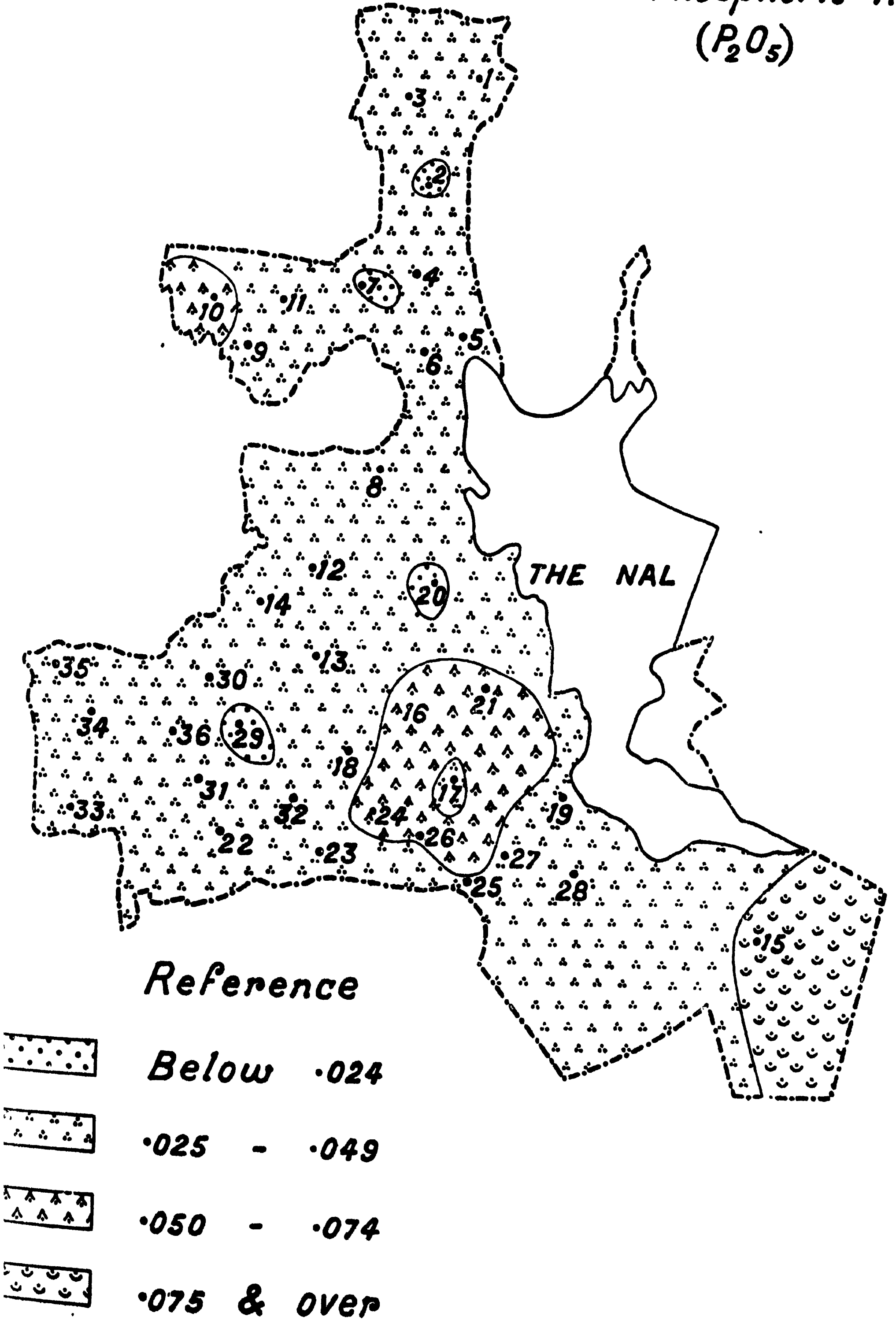
Such a deficiency is not indicated by the potash figures for there is a fair supply of this ingredient combined with appreciable amounts of available potash. Moreover, the lime in these soils will naturally liberate more available potash every year and maintain its supply for a sufficiently long period.

DISTRIBUTION OF NITROGEN.

From the distribution of nitrogen (Map 9), it may be seen that more than 85 per cent. of the area contains less than 0·04 per cent.,

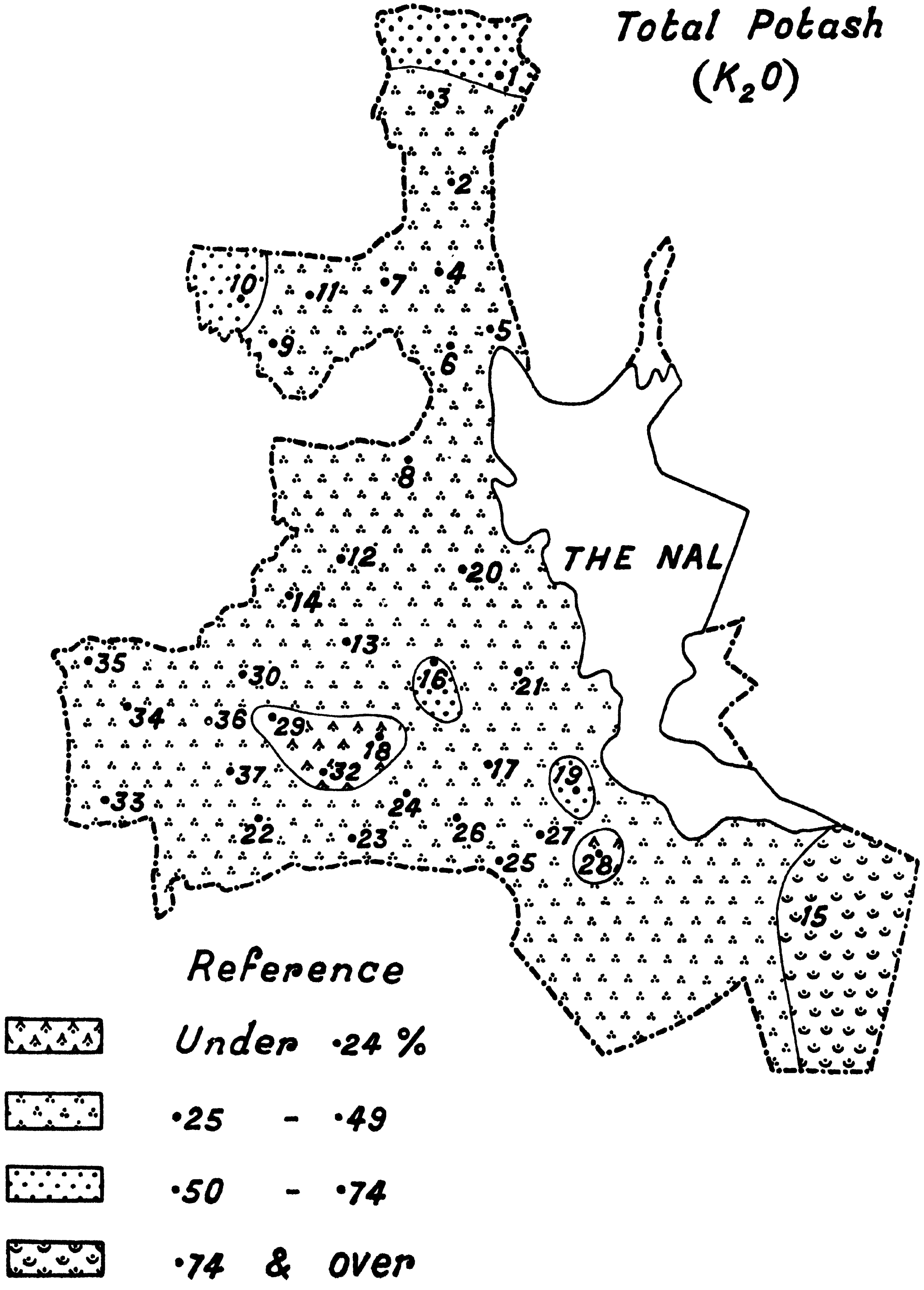
MAP No. 7

Distribution of total Phosphoric Acid (P_2O_5)



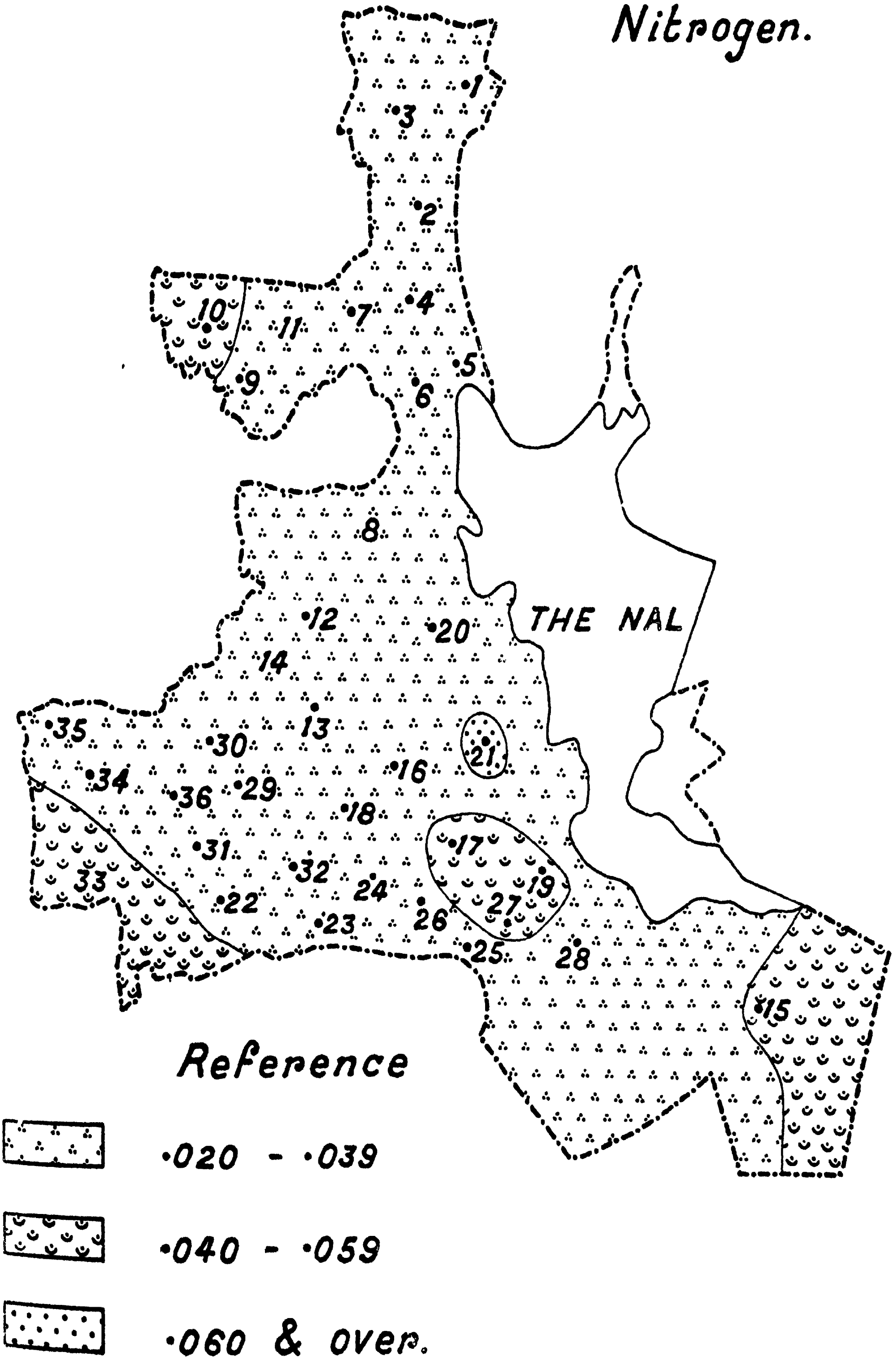
MAP No. 8

Distribution of Total Potash (K_2O)



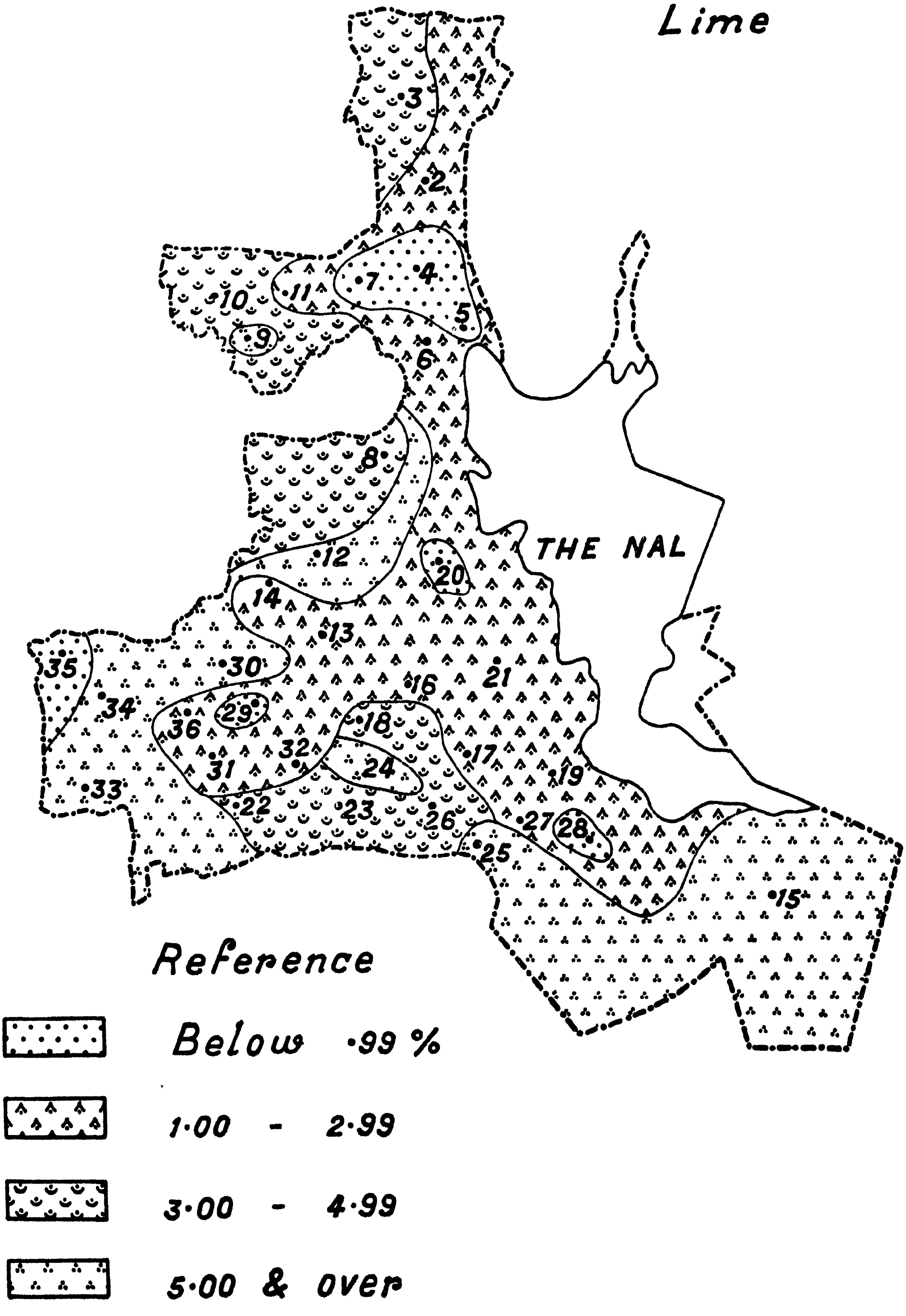
MAP No. 9

Distribution of Nitrogen.



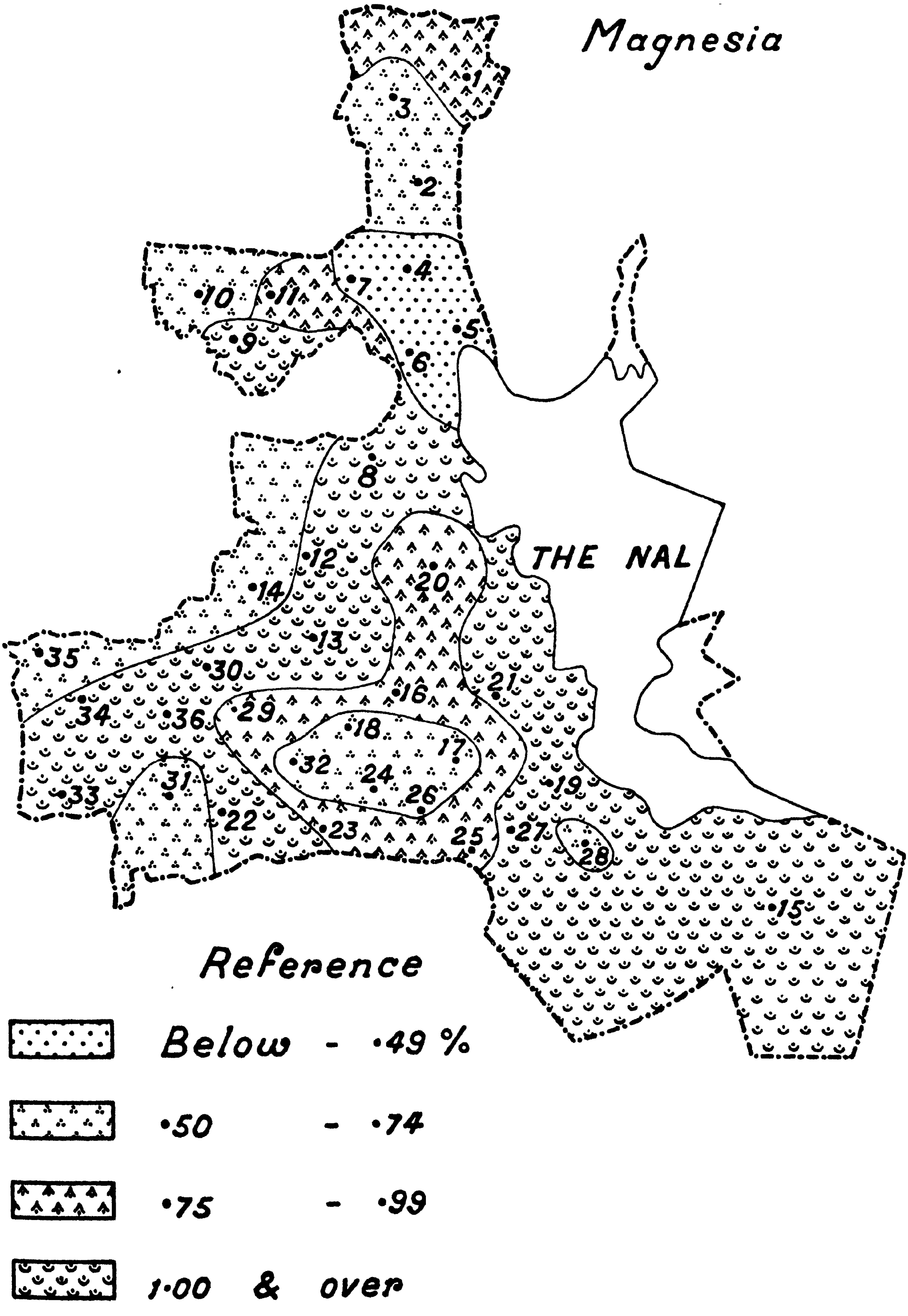
MAP No.10

Distribution of Lime



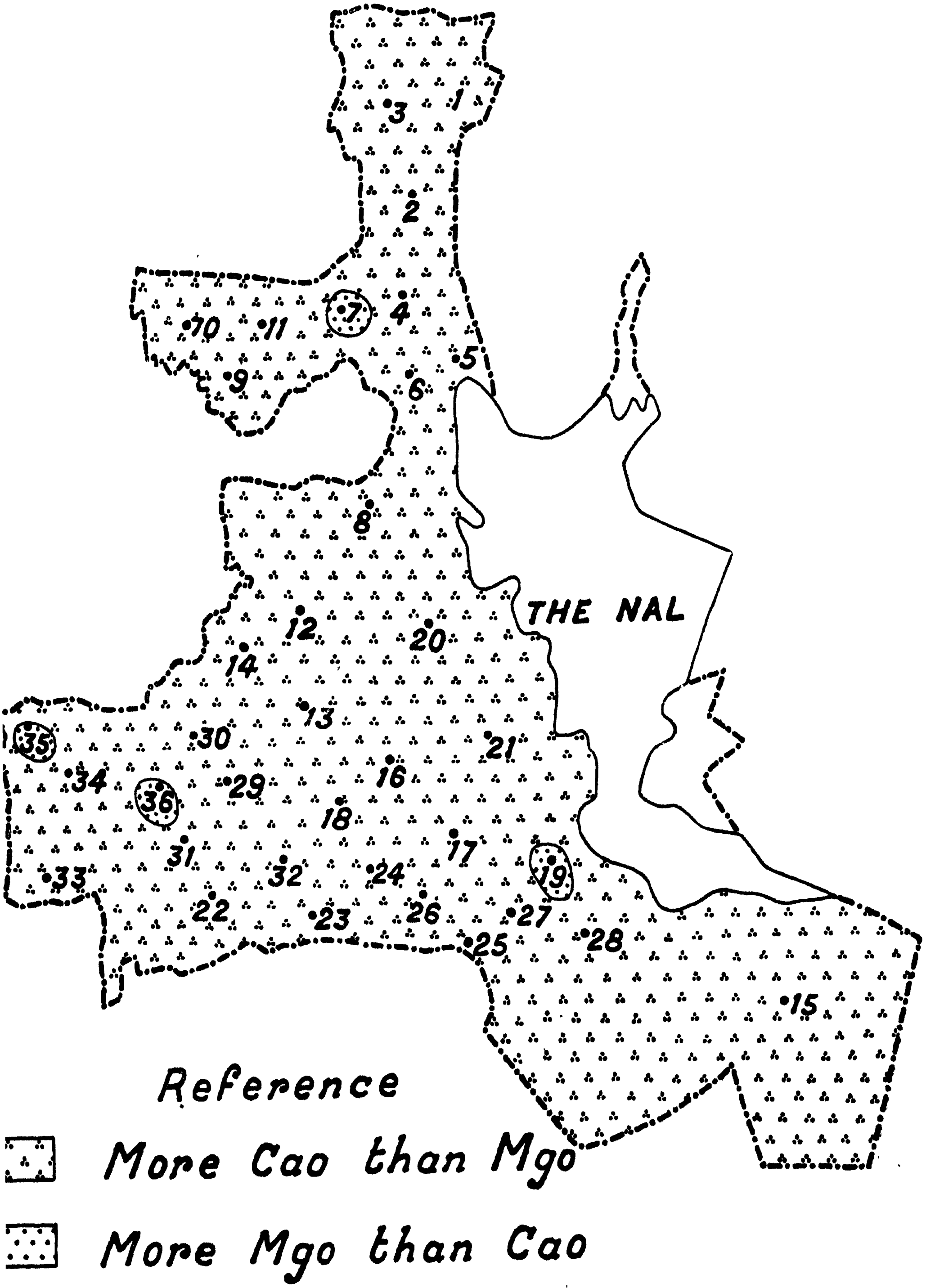
MAP No. 11

Distribution of Magnesia



MAP No. 12

Magnesia-Lime Ratio



suggesting high deficiency in nitrogen. The fact that no bulky organic manures, such as well preserved cattle manure, have been used in the cropping of these soils, points to this deficiency. Hence it is essential to lay out a systematic scheme of manuring whereby the soil requirements might be supplied. The preservation of cattle-manure combined with the utilisation of organic waste vegetation as suggested by Hutchinson (E.P., 219, 384) in the form of artificial cattle-manure may be suggested to supply this deficiency in the agricultural practices of the district.

The distribution of lime and magnesia (Maps 10 and 11) shows that 80 per cent. of the soils are well supplied with over one per cent. of lime. The supply of magnesia is far less than that of lime, the latter being always present in great excess. In the presence of much lime the organic matter of the soil is more readily broken down, hence the figures obtained furnish an additional argument for more extensive use of cattle-manure or other bulky organic manures in cropping. The magnesia-lime ratio is shown in Map 12.

It is interesting to observe that the foregoing observations are similar to those in most other parts of India.

MECHANICAL ANALYSIS OF SOILS.

Mechanical composition of the soils was determined by the sedimentation process using distilled water without subjecting the soils to any preliminary treatment with hydrogen peroxide or hydrochloric acid. Ammonia was not used to disperse the finer particles.

Puri (*Bull. Agri. Res. Inst. Pusa*, 1928, **175**, 11) has shown that treatment with hydrogen peroxide is quite unnecessary for Indian soils which usually contain very little humus ; hence the first treatment with hydrogen peroxide as recommended by the International method (*J. Agri. Sci.*, 1926, **16**, 123) was not adopted. It was also considered that treatment of soil with acid prior to the analysis was drastic and would materially alter the mechanical composition in the case of soils rich in lime such as those under study. Since the purpose of mechanical analysis is mainly to interpret the behaviour of soil as handled in the preparation of the land for raising crops, any preliminary treatment would naturally lead to the disintegration of the particles as they exist in nature. According to Hilgard (*Soil*, 1921, 288) " the disintegration of compound particles by means of acid as prescribed and practised should wholly change the physical nature of the soil by the breaking up of mechanical aggregates which in the usual course of tillage would remain intact " and hence a method in which such a change is not effected should be commended. The method followed in these studies and used in the study of Iraq soils

TABLE II.

Mechanical Composition of Soils.

Nalkantha District, Limbdi State.

Class of soil.	Lab. No.	Village	Stones per cent.	Fine soil contains: Fine gravel per cent.	Coarse sand per cent.	Fine sand per cent.	Silt per cent.	Fine silt per cent.	Clay per cent.
FIRST CLASS	1	Mothikathechi ...	22·22	10·01	21·10	20·72	14·70	24·57	7·30
	4	Nanikathechi ...	20·71	12·06	44·14	19·78	5·83	12·48	5·69
	8	Gadthal ...	29·77	9·06	20·29	21·19	11·95	26·92	9·62
	12	Bhagwanpura ...	25·71	4·35	10·40	21·24	17·34	30·68	13·49
	15	Dhigvijayagadh ...	19·50	1·07	0·81	11·34	25·72	37·29	22·83
	16	Ranagadh ...	33·29	9·61	15·59	14·00	7·74	33·99	18·15
	22	Dhanvana ...	34·08	7·94	25·84	16·10	10·81	26·48	11·14
	25	Mulbavla ...	16·58	4·47	20·91	16·64	11·17	32·93	10·20
	29	Pulvadi ..	28·69	9·82	25·83	25·01	11·48	18·29	9·71
	33	Rojhasar ...	34·15	6·24	20·28	20·75	18·52	25·80	7·68
SECOND CLASS	2	Mothikathechi ...	21·83	16·26	34·50	20·31	7·07	16·24	5·56
	5	Nanikathechi ...	22·21	14·71	44·50	16·35	4·38	13·90	5·73
	9	Gadthal ...	37·70	7·06	15·63	16·41	16·06	31·07	12·84
	13	Bhagwanpura ...	28·12	11·70	17·48	21·18	11·84	27·19	9·55
	17	Ranagadh ...	23·62	7·25	16·14	24·29	13·56	26·94	10·70
	23	Dhanvana ...	30·89	10·35	28·79	17·37	10·54	23·00	7·75
	26	Mulbavla ...	31·96	7·47	19·45	9·90	11·23	36·97	14·53
	30	Phulvadi ...	38·85	8·26	16·30	17·29	10·47	34·15	13·20
	34	Rojhasar ...	36·41	9·85	19·91	17·15	9·42	32·55	10·10
THIRD CLASS	3	Mothikathechi ...	40·13	29·42	25·04	27·62	4·00	9·84	1·29
	6	Nanikathechi ...	27·81	19·04	36·89	20·56	6·19	11·51	4·90
	10	Gadthal ...	22·53	7·85	20·91	24·36	14·06	22·46	10·29
	14	Bhagwanpura ...	29·10	21·92	31·52	26·32	4·68	13·19	2·96
	18	Ranagadh ...	26·63	26·30	35·92	20·90	3·73	8·58	3·36
	24	Dhanvana ...	27·02	7·36	19·72	28·26	15·97	21·06	6·94
	27	Mulbavla ...	37·20	10·96	17·36	10·02	12·13	29·86	16·79
	31	Phulvadi ...	28·58	8·10	23·63	20·07	10·24	27·64	8·13
	36	Rojhasar ...	33·98	10·86	28·51	16·33	7·62	26·27	9·42
FOURTH CLASS	7	Nanikathechi ...	28·92	23·21	47·37	11·96	4·05	9·25	4·35
	11	Gadthal ...	34·84	11·60	24·39	27·48	12·30	15·11	7·33
	28	Mulbavla ...	35·75	24·82	36·19	21·61	4·40	9·01	3·90
	32	Phulvadi ...	36·37	23·19	31·89	25·70	3·98	10·79	3·22
		RANAGADH							
	19	Paddy soil Komad ...	37·02	13·64	23·14	14·08	10·22	27·19	10·16
	20	,, Wari ...	29·27	18·91	37·98	17·66	4·55	8·62	10·93
	21	,, Experimental	26·54	16·74	25·05	17·75	6·63	24·93	7·42
		ROJHASAR							
	35	2nd Class Special ...	33·08	14·60	44·87	20·86	3·45	10·96	5·00

by Viswanath (private communication) has been found to suit the purpose of these investigations.

The general technique of sedimentation was followed for the separation of the various sizes of particles, using distilled water as the dispersing medium. The different fractions were steam dried and weighed, the results being expressed as percentages on the moisture-free soil for comparison. The results are given in Table II, arranged according to the existing classification of soils in the district. The figures for mechanical analysis of these soils are quite irregular and show the absence of any scientific basis for the present classification.

Hence an attempt was made to arrive at a workable scheme by grouping the results under two heads, namely, coarse and fine fractions. The coarse fraction represented the sum of the percentages of fine gravel, coarse sand and fine sand, particles of sizes ranging from 3 mm. to 0·04 mm. in diameter, and the fine fraction other grades ranging from 0·04 mm. to less than 0·002 mm. in diameter and including silt, fine silt and clay. This classification was made, because the above-mentioned finer fractions show, more or less, the same capacity for retaining moisture and manures, contributing considerably towards ease of cultivation and cropping. Although the coarse fractions also possess features in common amongst themselves, they have no such capacity for holding moisture or manurial ingredients as would constitute them a collective member in soil economy. Nevertheless, their presence in optimum amounts along with the finer grades of particles introduces factors quite favourable to the growth of plants. Hence this assumption seems well justified not only to the soil under study but may be of wider applicability to others. Further proof for this new classification is afforded by the behaviour of the soils in nature which agrees quite well with the depth of soils, their relationships to moisture and accordingly the types of crops now grown on them.

Table III shows the irregularity in the composition of different classes of soils according to the existing classification.

TABLE III.

Class of soil	Per cent. coarse fraction	Per cent. fine fraction
First	75·98	24·00
Second	50·36	48·58
Third	55·34	43·97
Fourth	82·62	17·31

The crops best suited to second class soil conditions are often indiscriminately raised on other classes indicating clearly the want of appreciation of principles underlying scientific cropping.

Table IV specifies the limits of variation between the two grades forming the basis of re-classification of soils and Table V shows actual average mechanical composition.

TABLE IV.

Class of soil	Depth of soil in feet	Limits in percentages of	
		Coarse fraction	Fine fraction
A	over 2	35 to 45	55 to 65
B	1½ – 2	45 to 55	45 to 55
C	1 — 1½	55 to 65	35 to 45
D	¾ – 1¼	70 to 80	20 to 30
E	¾ – 1	80 to 85	15 to 20

TABLE V.

Class of soil	Per cent. coarse fraction	Per cent. fine fraction	Character of soil
A	38·26	60·49	Clayey
B	50·74	48·01	Clayey loam
C	58·34	40·56	Loamy
D	75·88	23·59	Sandy loam
E	81·83	17·42	Sandy

Table VI gives the nature of the crops grown on the respective class of soil.

TABLE VI.

Class of soil	Nature of crops
A	Wheat (*Triticum vulgare*) and rarely cotton (*Gossypium Herbaceum*)
B	Cotton (*Gossypium Herbaceum*), Post monsoon Juar (*Sorghum vulgare*) and rarely Bajri (*Pennisetum typhoideum*)
C	Monsoon Juar (*Sorghum vulgare*), Bajri (*Pennisetum typhoideum*) and Banti (*Panicum crus-Galli* var. *Frumentaceum*)
D	Barley (*Hordeum vulgare*), Mhut (*Phaseolus aconitifolius*) and Mung (*Phaseolus mungo*)
E	Mhut (*Phaseolus aconitifolius*) and Mung (*Phaseolus mungo*)

It will be seen from the general nature of the crops grown on each class of soil that the character of their root-system and feeding habits are quite in keeping with the texture and moisture relationship of those soils. Thus the new scheme of classification agrees closely with the behaviour of soil in nature and the general system of cropping in the locality.

Under the suggested re-classification the soils have been placed under five groups A to E, each exhibiting definite characteristics. It will also be evident from the study of Table IV that the irregularities observed under the old system (cf. Table III) have been systematised and a more rational scheme has been evolved showing distinct gradation in the sizes of soil particles; class E being the most open textured containing more of coarse particles and class A the finest, with the highest amount of fine particles the other classes passing through the intermediate grades of sizes. With the help of these tables it is now possible to interpret the results of mechanical analysis and understand their behaviour in cropping.

The first two classes of soils, as their mechanical composition indicates, possess very high capacity for retaining moisture and hence they bear crops only after the cessation of the monsoon when the moisture conditions will be favourable for cropping. Besides, wheat always requires a very fertile field for its growth and hence in these two soils wheat and cotton are the commonest crops grown. Whereas the other three classes of soil bear such crops as have a very shallow root-system principally during the monsoon period when most of their vegetative phase is passed and they come to maturity before all moisture is lost from the soil.

Additional support is afforded by the results of average chemical composition of the corresponding groups of soil as shown in Table VII, wherein an abstract of the average mechanical composition of those groups is also given with a view to bringing out the correlation between them.

TABLE VII.

(A). *Average mechanical composition of soils.*

Serial No.	Class of soil	FINE SOIL CONTAINS							
		Coarse fraction			Aggregate coarse fraction	Fine fraction			Aggregate fine fraction
		Fine gravel per cent.	Coarse sand per cent.	Fine sand per cent.		Silt per cent.	Fine silt per cent.	Clay per cent.	
1	A	7·13	15·66	15·47	38·26	13·98	32·53	13·98	60·49
2	B	8·84	20·77	21·13	50·74	12·45	26·03	9·53	48·01
3	C	10·00	25·45	22·89	58·34	11·58	20·75	8·23	40·56
4	D	15·33	40·98	19·57	75·88	5·19	13·02	5·38	23·59
5	E	24·81	34·66	22·36	81·83	4·14	10·10	3·18	17·42

(B). *Average chemical composition of soils.*

Serial No.	Class of soil	Nitrogen per cent.	Lime per cent.	Magnesia per cent.	Phosphoric acid, P_2O_5 per cent.		Potash, K_2O per cent.	
					Total	Available	Total	Available
1	A	0·036	5·95	1·17	0·054	0·0045	0·50	0·0328
2	B	0·036	3·08	1·05	0·040	0·0050	0·43	0·0232
3	C	0·030	2·72	0·88	0·036	0·0048	0·32	0·0272
4	D	0·029	1·27	0·52	0·031	0·0141	0·37	0·0194
5	E	0·030	3·75	0·55	0·028	0·0050	0·25	0·0157

There is a distinct gradation in the fertility of these soils, as shown by the figures of chemical analysis, agreeing closely with their mechanical composition. The correlation existing between the mechanical and the chemical composition of these soils requires some explanation. In this connection it will be of interest to examine the average composition starting from class E, when the peculiarities in the results will become evident. It is known that a large percentage of the coarse fraction with a proportionate decrement in the finer is associated with low figures for the reserve phosphoric acid and potash apart from other mineral constituents; for the reserves are derived more from the finer grades than from the coarser, the latter being composed principally of sand particles. This inference is borne out by the chemical analysis; with the progressive increase in the finer grades and a corresponding gradual decrease of the coarser fraction, an increase in total phosphoric acid and potash is found. Hence the total phosphoric acid and potash in class A soils is nearly double that of class E. Owing to the precipitation of the available phosphate by other mineral constituents such as iron, alumina, lime and magnesia, whose percentage increases in the finer grades, the latter, although containing a useful phosphate-reserve, do not make this so freely accessible to crops. The stationary aspect of available phosphate is thus explained. This influence is not noticed with available potash which shows a marked increase with increasing fineness; access of lime will have a beneficial influence in liberating potash from minerals containing it.

No significant change is noticeable in the nitrogen figures as these depend less on the size of particles than on organic matter in the soil. The fact that these soils have not been manured for over a long period with bulky organic manures easily explains the constancy in the nitrogen values of all soils.

It has been definitely shown in the case of this soil survey that a classification of soils on the basis of their texture will naturally in generality of soils closely correspond to their chemical composition which, in other words, signifies their fertility. This observation may be of wide applicability to other soils.

SUMMARY.

The results of the soil survey of the Nalkantha district, Limbdi State, comprising the chemical and mechanical analysis of the soils are discussed.

The chemical analysis has brought out the great deficiency in phosphoric acid and nitrogen in the soils of the district indicating the need for phosphatic and nitrogenous manures. In this connection the utilisation of bones and the use of well preserved cattle-manure, along with the proper use of all waste vegetation found in abundance, in the practice of agriculture is strongly recommended.

The supply of potash and lime in these soils is quite adequate. Thus the chemical analysis has borne out the manurial requirements of the soils which may be mentioned as a common feature of almost all the Indian soils so far examined.

The mechanical composition of these soils shows a great range of irregularity when viewed by the existing method of classifying soils, indicating absence of a scientific basis for this classification.

A new scheme has been proposed for the scientific classification of soils based upon their physical texture as shown by the results of mechanical analysis, and this agrees closely with the general behaviour of soils in nature.

The correlation shown by the results of chemical analysis of the corresponding groups of soils classified on the above basis lends further confirmation to the soundness of the principles advocated under the new scheme.

As the new basis clearly indicates the fertility of soils with special reference to the tract under discussion, it may prove of wide applicability to other soils.

I wish to take this opportunity of expressing my grateful thanks to Prof. Roland V. Norris for the keen interest he has taken in this work.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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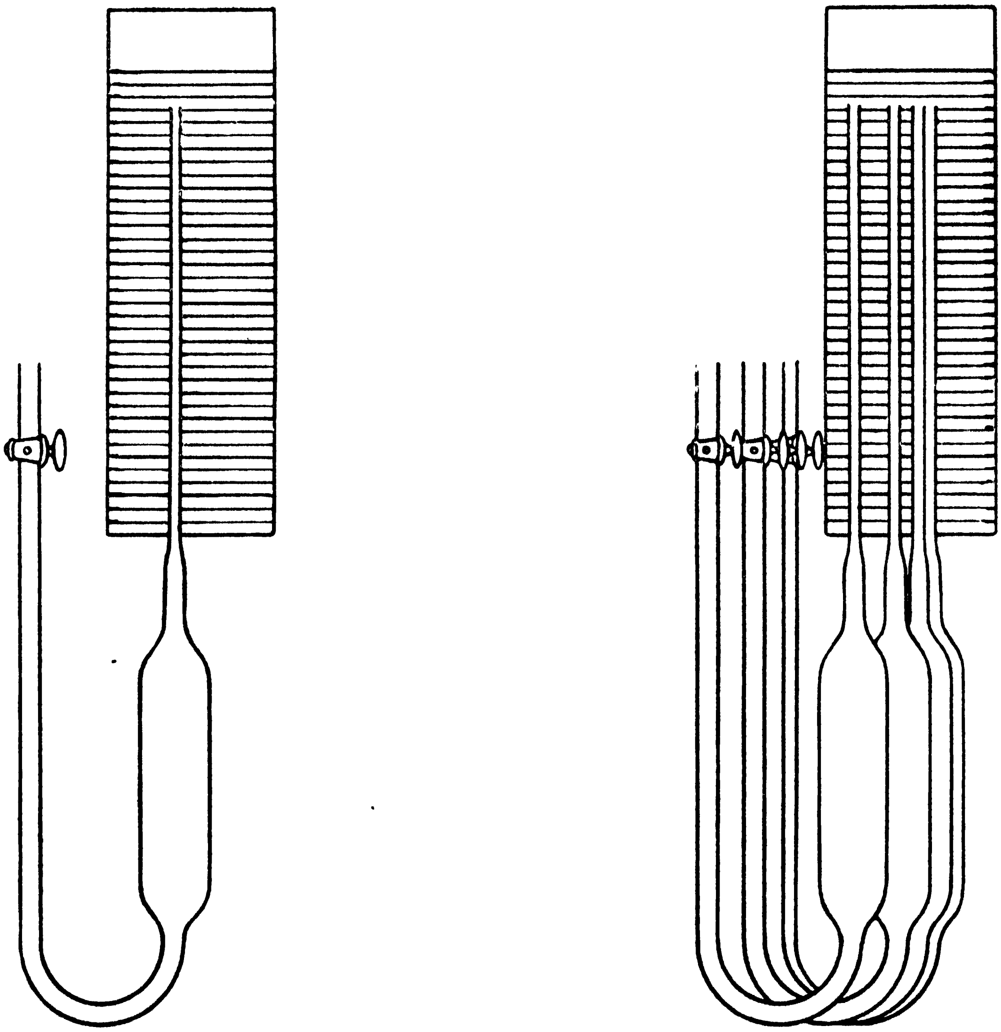

Fig. 1

(a)

(b)

DILATOMETRIC STUDIES IN ENZYME ACTION.*

By M. Sreenivasaya and B. N. Sastri.

The changes in volume accompanying enzyme actions are measurable and can therefore be studied with the aid of a dilatometer. Koelichen (*Z. physikal. Chem.*, 1901, **33**, 154) utilised the volume change to determine the velocity of decomposition of acetonyl alcohol in presence of sodium hydroxide. Benrath (*Z. physikal. Chem.*, 1909, **67**, 501) has made a study of the density changes of reacting liquid systems such as the inversion of sucrose and formation and hydrolysis of esters. Van't Hoff (*Sitzungsber. preuss. Akad. Wiss.*, 1910, **34**, 963) extended its application to a study of the enzymic synthesis of glucosides. Galeotti (*Z. physikal. Chem.*, 1911, **76**, 105) investigated dilatometrically the inversion of sucrose, the saponification of ethyl acetate, and the hydrolysis of starch, peptone and proteins by acids and in some cases by enzymes. Later (*Z. physikal. Chem.*, 1912, **80**, 241) he studied the conditions of synthesis of esters and fats brought about by pancreatic extract, employing the dilatometer. In spite of the lead given by the above authors, the dilatometer does not appear to have been extensively employed in the investigation of enzymes, in spite of its simplicity and manipulative elegance and the accuracy of the results that can be attained.

THE DILATOMETER.

The instrument consists essentially of a bulb nearly 50 c.c. in capacity. To one end is fused a tap, whilst the other end is connected to a capillary. The whole is bent into a U-form [see Fig. 1(*a*)] the capillary-bearing bulb and the tap forming the two arms. Several such dilatometers can be used simultaneously by fixing them all by means of their capillaries to a suitable clamp [Fig. 1 (*b*)]. A common scale, fixed behind the set of capillaries, allows the easy reading of the meniscus.

Agitation of the reaction mixture which is necessary in the case of insoluble enzymes like lipase, may be carried out by introducing a few glass beads into the bulb, before fusing on the tap, and mechanically shaking the dilatometer. A more elegant method is to introduce into the bulb a platinum-plated stirrer which can be worked electromagnetically.

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* Reprinted from the *Biochemical Journal*, 1929, 23, 975.

EXPERIMENTAL.

The dilatometer is thoroughly washed successively with alcoholic potassium hydroxide, chromic acid, distilled water, alcohol and finally with ether, and then dried. The taps are carefully greased and a small reservoir of about 75 c.c. capacity is attached to the tap end of the instrument by means of pressure tubing. By opening the tap and applying gentle suction at the capillary end, filling can be smoothly effected without the introduction of air bubbles.

The operation is carried out with the dilatometer and the reaction mixture completely immersed in the thermostat. Filling takes less than 5 minutes and the first reading can easily be recorded within that period. The temperature variation of the thermostat employed was ±0·001° and a control dilatometer which was always used served as an effective indicator of the constancy of the temperature maintained throughout the experiment.

The capacity of each of the dilatometers employed was determined and the capillaries were carefully calibrated in the usual way. A dilatometric study of the following enzyme reactions was carried out: taka-diastase on starch, invertase on sucrose, emulsin on salicin, tannase on methyl gallate and amidase on asparagine. In the case of diastase, the method has been extended to a study of the influence of salts and also to a determination of the rate of liquefaction. Every one of the dilatometrically investigated reactions has been simultaneously followed by an entirely independent method to establish the accuracy of the method.

TABLE I.

Hydrolysis of starch by taka-diastase.

Materials : Lintner's soluble starch, 5 per cent. solution; taka-diastase (Parke Davis), 1 per cent. solution.

Mixture employed: 220 c.c. starch solution + 5 c.c. enzyme.

Temperature of thermostat: 30·0°.

Time	Dilatometer readings, mm.		Maltose value c.c $KMnO_4$
	Control	Experimental	
30 mins.	108	122·5	4·50
45 ,,	108	118·5	5·90
65 ,,	108	111·5	7·30
95 ,,	108	105·0	9·35
132 ,,	108	96·5	11·65
220 ,,	108	80·5	15·80
295 ,,	108	69·5	18·20
364 ,,	108	61·5	25·55
24 hrs.	108	13·0	29·50
30 ,,	108	8·0	31·40

Fig. 2

Hydrolysis of Starch by Taka Diastase.

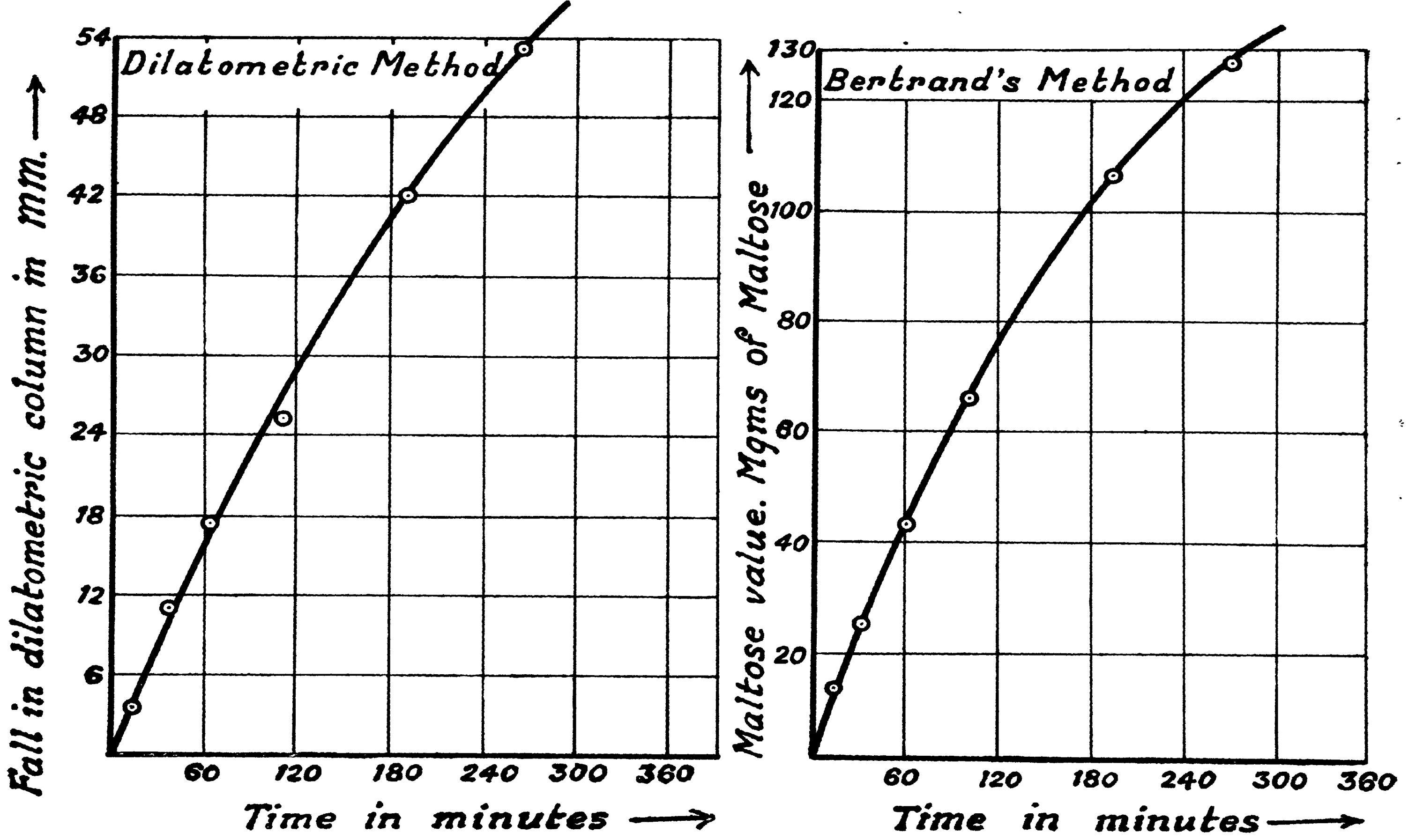
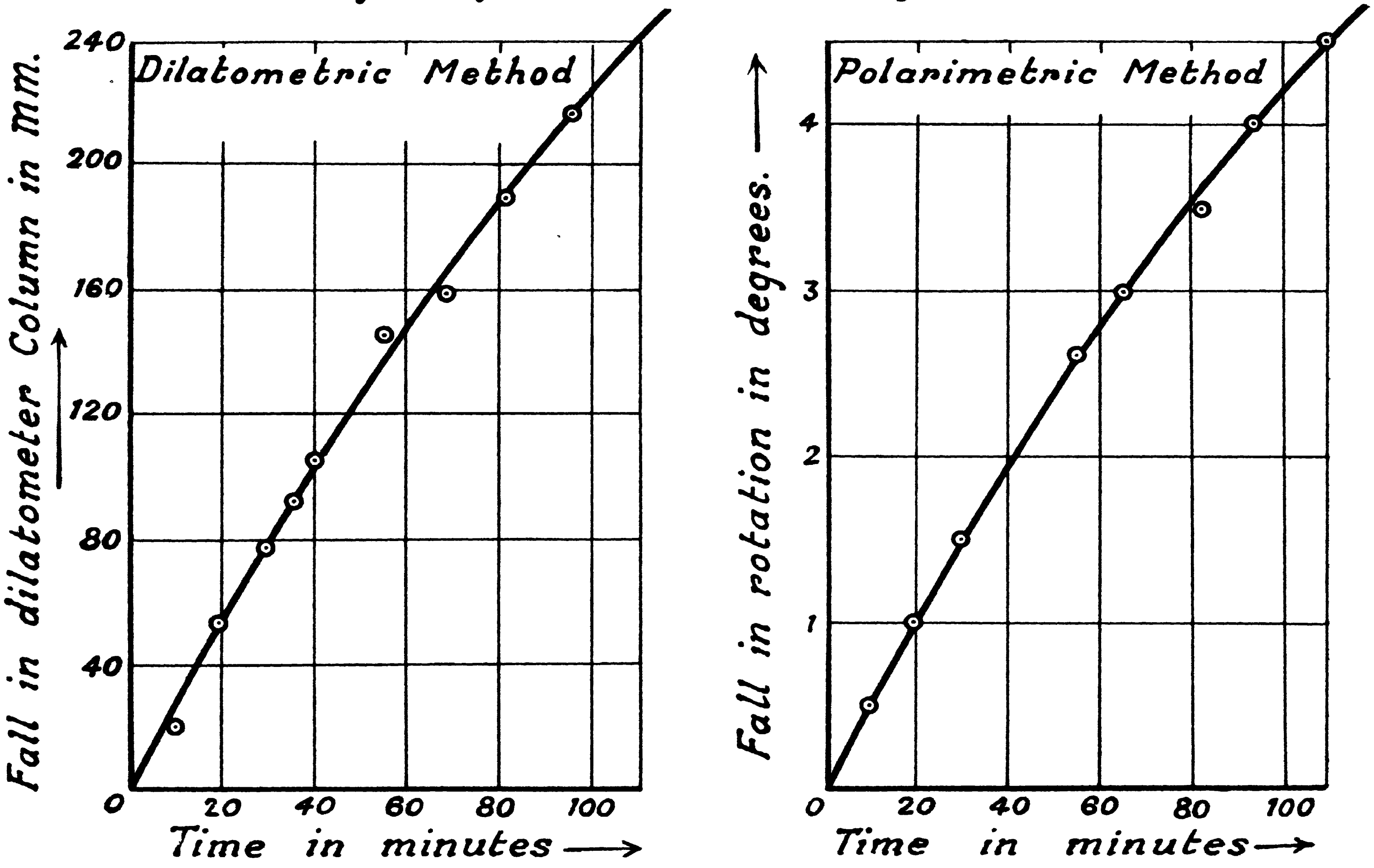


Fig. 3

Hydrolysis of Sucrose by Invertase



10 c.c. of the reaction mixture were employed each time for Bertrand's estimation of maltose. Strength of $KMnO_4$: 1 c.c. = 10 mg. Cu (see Fig. 2).

TABLE II.

Action of invertase on sucrose.

Materials: sucrose solution, rotation + 5°: invertase, activity −3·45°*.

Solutions employed: 300 c.c. sucrose solution + 3 c.c. enzyme; 300 c.c. sucrose solution + 3 c.c. boiled enzyme.

Temperature of thermostat: 30·0°.

Time	Dilatometer readings, mm. Control	Dilatometer readings, mm. Experimental	Polarimetric readings
9 mins.	385	232·5	4·50°
19 „	385	252·5	4·00°
28 „	385	283 0	3·51°
38 „	385	308·0	3·02°
44 „	385	324·0	—
49 „	385	339 0	1·93°
65 „	385	378 5	—
78 „	385	411·0	0·95°
91 „	385	441·0	—
104 „	385	469·0	0·51°
114 „	385	490·2	—
120 „	385	500·0	0·04°

The two curves are superimposable (Fig. 3).

TABLE III.

Action of emulsin on salicin.

Materials: 1 per cent. salicin solution (Kahlbaum's); 0·5 g. enzyme (B.D.H.) in 10 c.c. water.

---

* The fall in rotation brought about by unit volume (1 c.c.) of the enzyme acting on 20 c.c. of a 10 per cent. solution of sucrose at 25° for 30 mins., measures the activity of the enzyme preparation (Sastri and Norris, *J. Indian Inst. Sci.*, 1928, **11A**, 6).

Solutions employed: experimental, 175 c.c. salicin solution + 5 c.c. enzyme; control, 175 c.c. salicin solution + 5 c.c. boiled enzyme.

Temperature of thermostat: 30·0°.

Time	Dilatometer readings, mm. Control	Dilatometer readings, mm. Experimental	$KMnO_4$ c.c.
22 mins.	214	406	—
34 ,,	214	392·5	—
75 ,,	214	363	14·55
94 ,,	214	354·5	—
120 ,,	214	347	17·20
155 ,,	214	340	—
157 ,,	214	—	18·50
207 ,,	214	334	—
213 ,,	214	—	19·70
267 ,,	214	330	—
273 ,,	214	—	20·00
332 ,,	214	325	—
387 ,,	214	—	20·10
392 ,,	214	324·5	—

The two curves are superimposable (Fig. 4).

TABLE IV.

Action of cholam amylase on starch paste.

Materials: 1 per cent. starch paste (Kahlbaum's potato starch); 1 per cent. enzyme solution.

Solutions employed: experimental, 400 c.c. starch paste + 16 c.c. enzyme; control, 100 c.c. starch paste + 4 c.c. boiled enzyme.

Temperature of thermostat: 30·0°.

Time	Dilatometer readings, mm. Control	Dilatometer readings, mm. Experimental	Viscometer readings. Time in secs.
12 mins.	206	285·0	—
32 ,,	206	285·0	—
45 ,,	206	—	118·0
47 ,,	206	287·0	—
72 ,,	206	288·5	—
75 ,,	206	—	116·0
105 ,,	206	292·1	—
110 ,,	206	—	114 5
185 ,,	206	—	110·5
260 ,,	206	299·0	—
265 ,,	206	—	107·5
320 ,,	206	299·5	106·2
440 ,,	206	303 0	105·4
720 ,,	206	307·5	105·0

(See Fig. 5.)

Fig. 4

Hydrolysis of Salicin by Emulsin

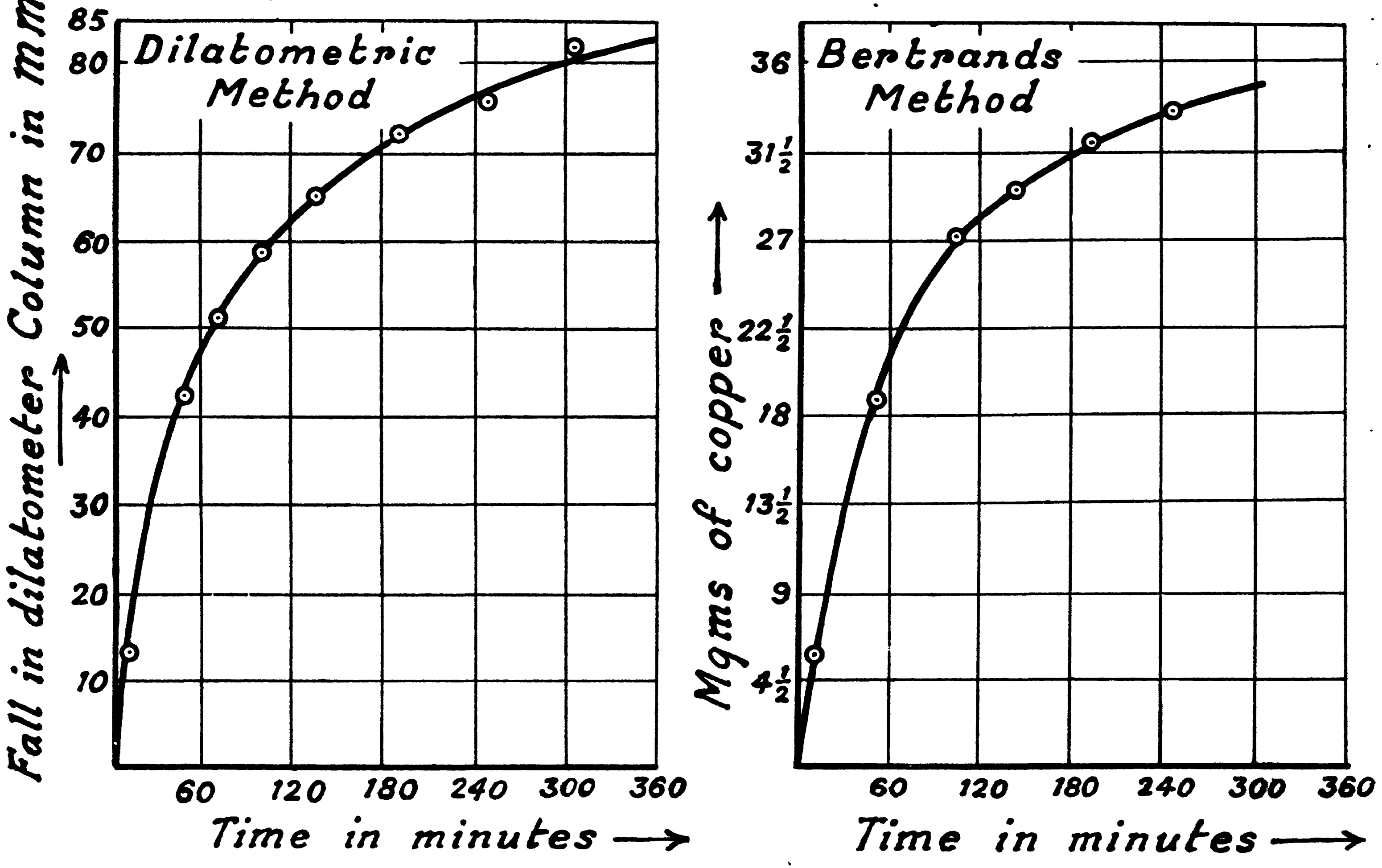
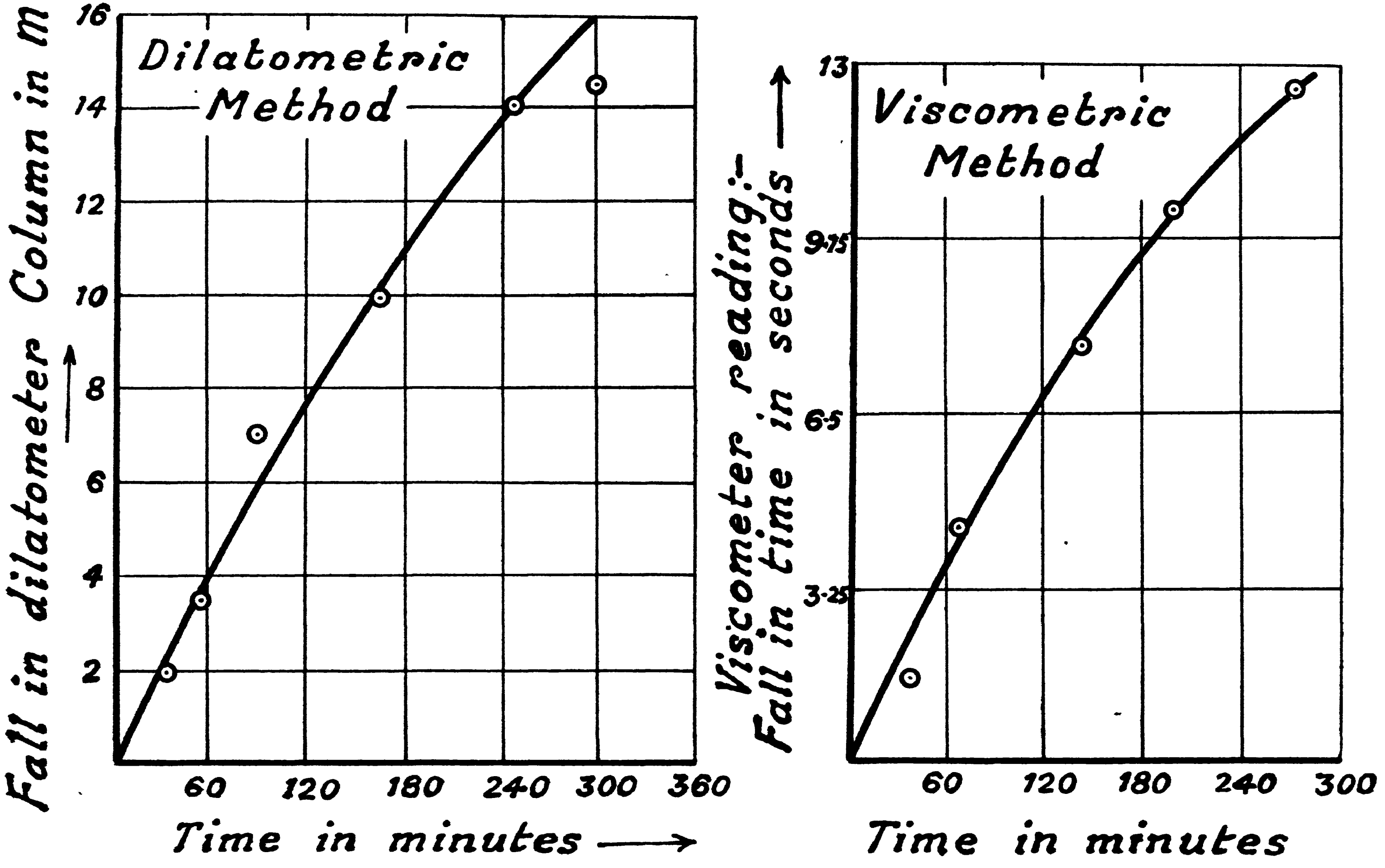


Fig. 5

Liquefaction of starch Paste by Cholam Amylase



Hydrolysis of methyl gallate by Tannase

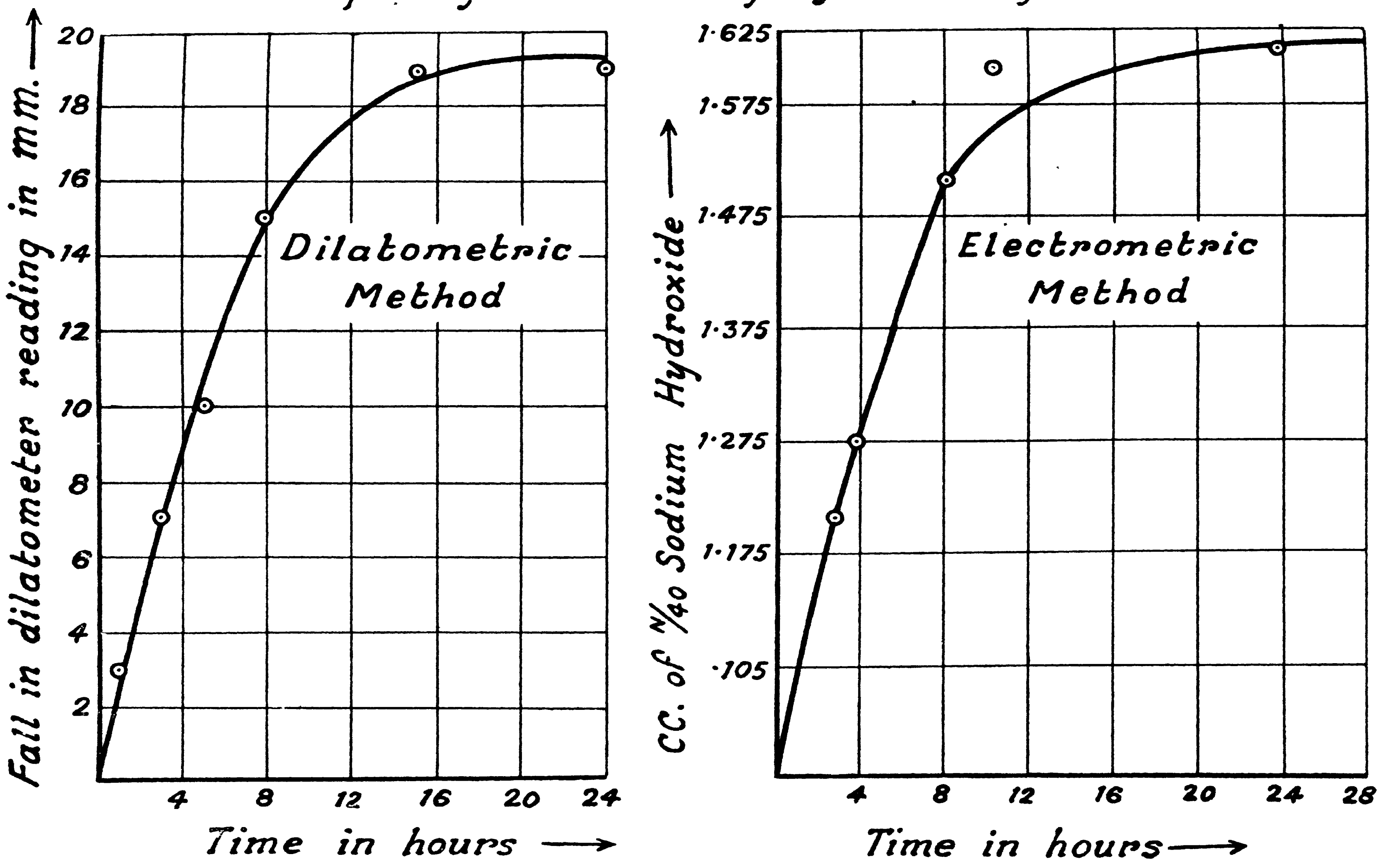


Fig. 7

Influence of Potassium Nitrate on the hydrolysis of Starch by T. Diastase

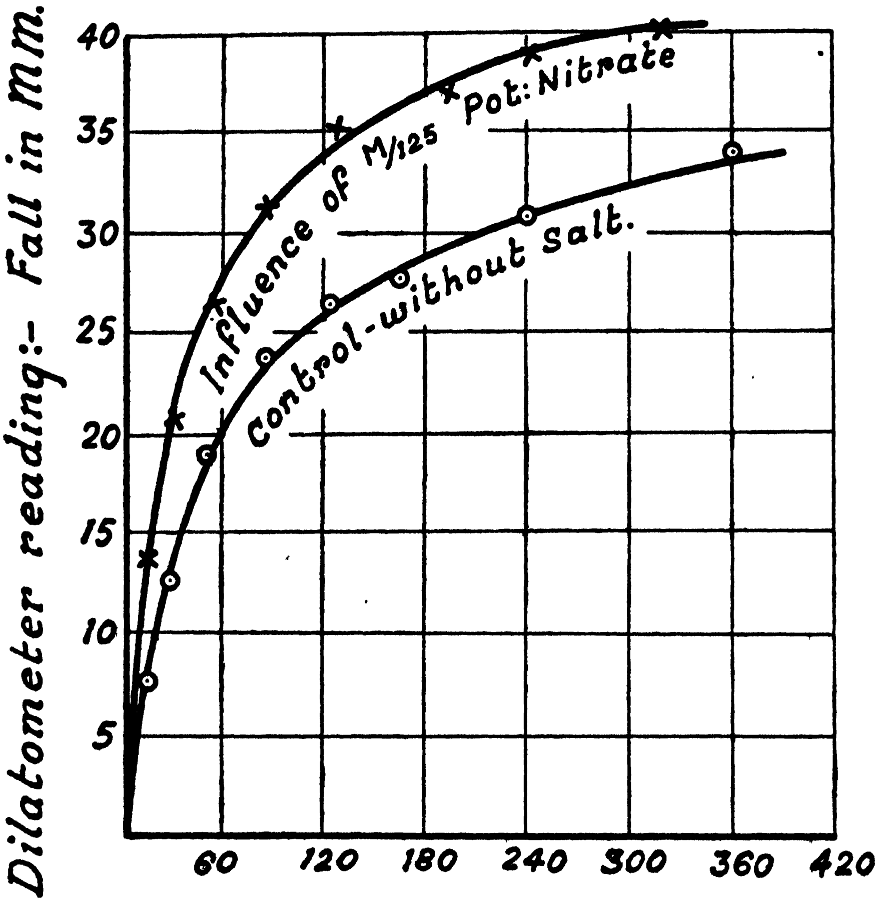


TABLE V.

Hydrolysis of methyl gallate by tannase.

Materials: 0·541 per cent. solution of methyl gallate; enzyme extract, 10 g. mould in 150 c.c. water.

Solution employed: experimental, 80 c.c. methyl gallate solution + 20 c.c. enzyme extract; control, 80 c.c. methyl gallate solution + 20 c.c. boiled extract.

Temperature of thermostat: 33·0°.

	Dilatometer readings, mm.		Electrometric titration
Time	Control	Experimental	c.c. *N*/40 NaOH
0 hrs.	201	202·5	0·975
1 ,,	201	199·5	—
3 ,,	201	195·5	—
4 ,,	201	—	1·275
5·25 ,,	201	192·3	—
8 ,,	201	187·5	—
10 ,,	201	184·5	—
10·5 ,,	201	—	1·600
15 ,,	201	183·3	—
24 ,,	201	183·2	1·625
30 ,,	201	—	1·625

(See Fig. 6.)

TABLE VI.

Influence of salts on enzyme action.

Solutions employed: experimental, 200 c.c. of 2 per cent. soluble starch, 10 c.c. of 1 per cent. taka-diastase and 10 c.c. of *M*/5 potassium nitrate in 250 c.c. control, without salt.

	Dilatometer readings, mm.	
Time	Control	Experimental
22 mins.	415·0	405·0
37 ,,	407·5	391·0
52 ,,	402·0	384·0
75 ,,	396·0	378·5
105 ,,	391·5	374·0
145 ,,	388·5	370·0
185 ,,	387·5	—
210 ,,	—	367·5
260 ,,	384·0	—
265 ,,	—	366·0
340 ,,	—	365·0
385 ,,	381·5	—
19·5 hrs.	363·5	356·0

(See Fig. 7.)

DISCUSSION.

A study of the tables and graphs demonstrates the closeness of the results obtained by the dilatometer and the methods generally adopted. In many cases, the dilatometric method has proved more satisfactory and less tedious. The estimation of sugars resulting during enzyme hydrolysis or the electrometric titration of acidities produced during the hydrolysis of methyl gallate and other esters is certainly more cumbersome and tedious than taking readings of the dilatometer at intervals.

The influence of salts like potassium nitrate on diastase has been studied. The study is being extended to other ions and colloids such as agar-agar, silicic acid, etc.

Limitations of the method.—In cases where the volume change is not pronounced, the method offers no special advantage over other methods. Again, the instrument cannot be employed in a study of enzyme reactions which involve the liberation of gases. In the course of an investigation of a preparation of amidase from *Aspergillus niger* on asparagine, no volume change was noticeable. The ammonia liberated during the enzymic cleavage of amides dissolves in the reaction mixture producing an increase in volume which is opposed to the fall due to hydrolysis. In such cases, accompanied by opposing changes of volume, this method is inapplicable.

SUMMARY.

1. A convenient form of dilatometer for the study of enzyme action is described.

2. The hydrolytic action of diastase, invertase, emulsin, amidase and tannase on their respective substrates has been studied.

3. Limitations of the method are discussed.

Our thanks are due to Mr. V. N. Patwardhan, for supplying us with a preparation of cholam amylase, and to Mr. P. D. Dalvi for making a dilatometric study of tannase.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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I. STUDIES IN NEUTRAL SALT ACTION.

Part I.—Diastase.

By D. Narayanamurti.

It is known that neutral salts are often of importance in enzyme action. Enzymes being colloidal systems will naturally respond to the action of electrolytes. Starkenstein, Bierry and Giaja (*Compt. rend.*, 1906, **143**, 300) and others have shown that animal diastase is inactive in the absence of neutral salts. Höber (*J. Gen. Physiol.*, 1928, **8**, 265) attributes the ionic antagonism observed by him in enzyme systems to the colloidal part of the complex. Neuschloss (*Kolloid Z.*, 1920, **27**, 292) has drawn attention to the great similarity between enzymes and lecithins. It is evident that electrolytes might act on enzymes in various ways, namely, change in degree of swelling, electro-kinetic potential, adsorption power, degree of dispersion, surface conductance, etc., and as remarked by Bayliss it is difficult to formulate statements of general application. This communication forms part of an extended series of investigations undertaken by the author to elucidate the mechanism of neutral salt action.

Influence of neutral salts on malt diastase.—There has been much controversy regarding the action of neutral salts on malt diastase. Hahn and Hapruder (*Z. Biol.*, 1919, **71**, 287, 302) distinguished the effect of neutral salts on malt and salivary diastase at three ranges, (*a*) optimal P_H, (*b*) acid side of optimal P_H and (*c*) alkaline side of optimal P_H. In buffered solutions salts were without any effect at the optimum P_H, on the acid side at great dilution of the buffer and neutral salt, acceleration was caused; at higher concentrations of buffer, inhibition was observed. On the alkaline side of the optimal P_H at great dilution of buffer and salt pronounced activation was caused, at higher concentration this was less marked, but inhibition was never observed. The decrease of the activating effect can, at higher buffer concentration, pass into inhibition in the case of nitrates. They further observed that the optimum P_H is not changed (cf. however Sherman, *J. Amer. Chem. Soc.*, 1928, **50**, 2532), that the enzyme wanders to the cathode at the optimum P_H and that on the addition of salt it wanders to the anode, when the isoelectric point is shifted to the acid side. Hence they consider that the electrical nature of the enzyme is of no consequence, and that the electrochemical dissociation theory of Michaelis finds no application to malt and salivary diastase. Fricke and Kaja (*Ber.*, 1924, **57**, 310, 313) found that addition of neutral salts inactivated their electrodialysed enzyme, Patwardhan and Norris (*J. Indian Inst. Sci.*, 1928,

11A, 127) found activation with malted cholam diastase (alcohol precipitated) and inactivation with unmalted cholam diastase at high salt concentration. Eadie (*Biochem. J.*, 1926, **20**, 1016) observed slight activation at salt concentrations of about 1·62*N*. Narayanamurti and Norris (*J. Indian Inst. Sci.*, 1928, **11A**, 134) noticed inactivation with electro-dialysed cholam diastase at the concentration of salt found most beneficial by Patwardhan and Norris.

In view of the above observations it was deemed necessary to investigate the matter in more detail and the results so far obtained are of sufficient interest to place on record.

EXPERIMENTAL.

The enzyme was prepared by Euler's method (*Z. physiol. Chem.*, 1920, **112**, 193) from cholam malt the dialysis in collodion bags against flowing distilled water being continued for ten days. A distinct advance was also made by using electrolyte-free amylose prepared according to Samec (*Kolloid Beihefte*, 1920, **12**, 281) in place of ordinary starch as substrate. The sodium chloride used was Kahlbaum's analytical reagent dissolved in conductivity water. All reactions were carried out in silica vessels washed with hot dichromate-sulphuric acid mixture and steamed before use.

Order of adding components in preparing the reaction mixture.—It is quite evident from the results shown in Fig. I., that the order of addition has a marked influence on the activity of the enzyme. The neutral salt was most pronounced in action when added to the enzyme before the amylose, thus recalling similar behaviour with colloidal systems. This is to be expected from the nature of diastase action, there being ample evidence to show that enzyme action ought to be considered as a case of contact catalysis. It is well known that adsorption has much to do with contact catalysis, and Frumkin (*Nature*, 1926, **117**, 790; *Biochem. Z.*, 1927, **182**, 220) has investigated the influence of electric fields on the adsorption of neutral molecules. His experiments indicate that adsorption is greatest when the adsorbent is almost uncharged. He found maximum adsorption at a small negative charge in the case of a mercury surface and a small positive charge in the case of a silver surface. Later work by Obretschewa (*Biochem. Z.*, 1929, **207**, 25) however, has shown that the relations are not so simple and that the concentration of the adsorbate must also be taken into account. With higher concentrations the maximum is slightly diverted from the isoelectric point. The adsorption depends not only on the electrical nature but also on the degree of dispersion of the adsorbent. This indicates that the decomposition of the neutral

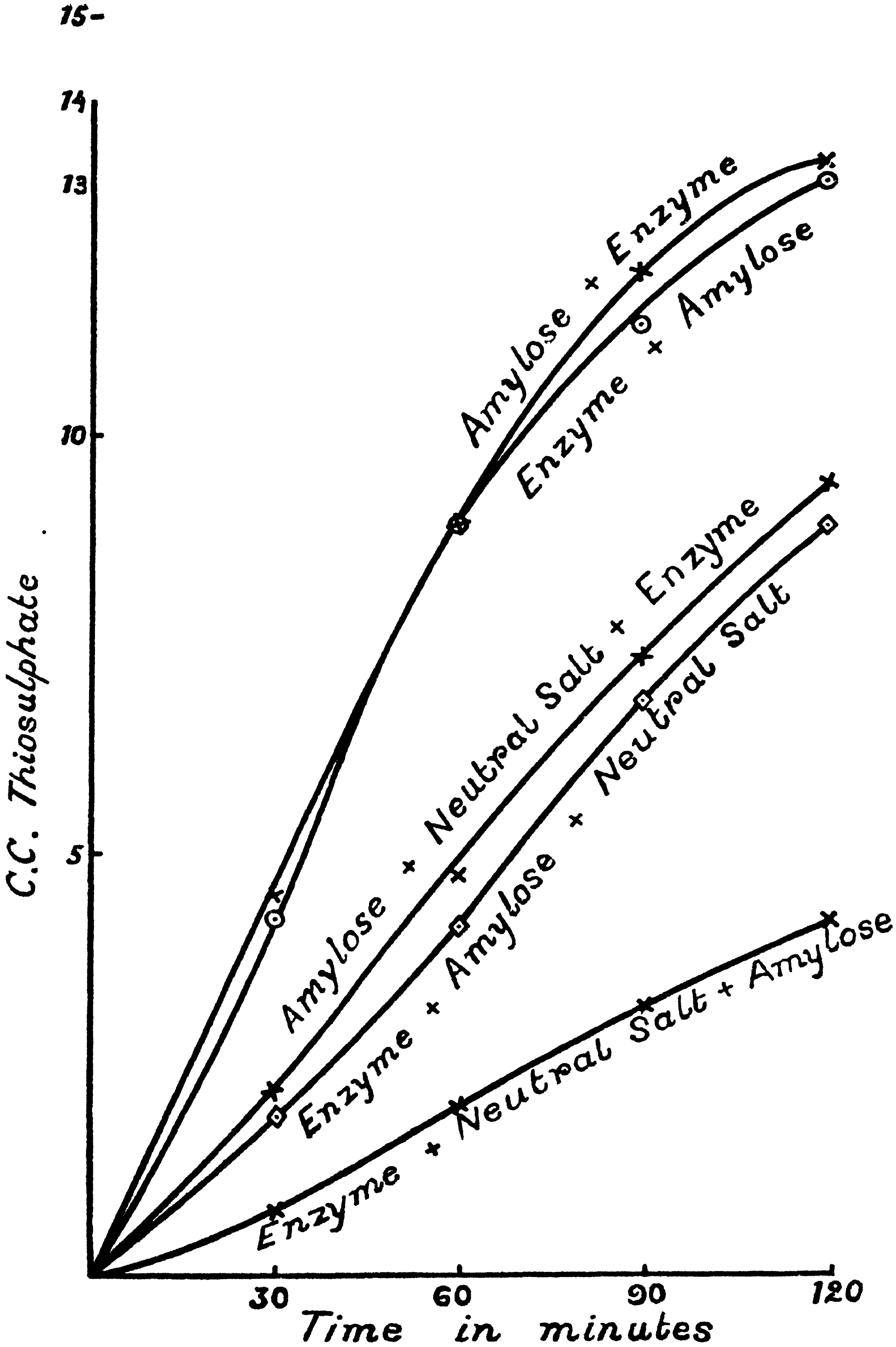
15-
14
13
10
5
C.C. Thiosulphate
30
60
90
120
Time in minutes
Amylose + Enzyme
Enzyme + Amylose
Amylose + Neutral Salt + Enzyme
Enzyme + Amylose + Neutral Salt
Enzyme + Neutral Salt + Amylose

Fig. I

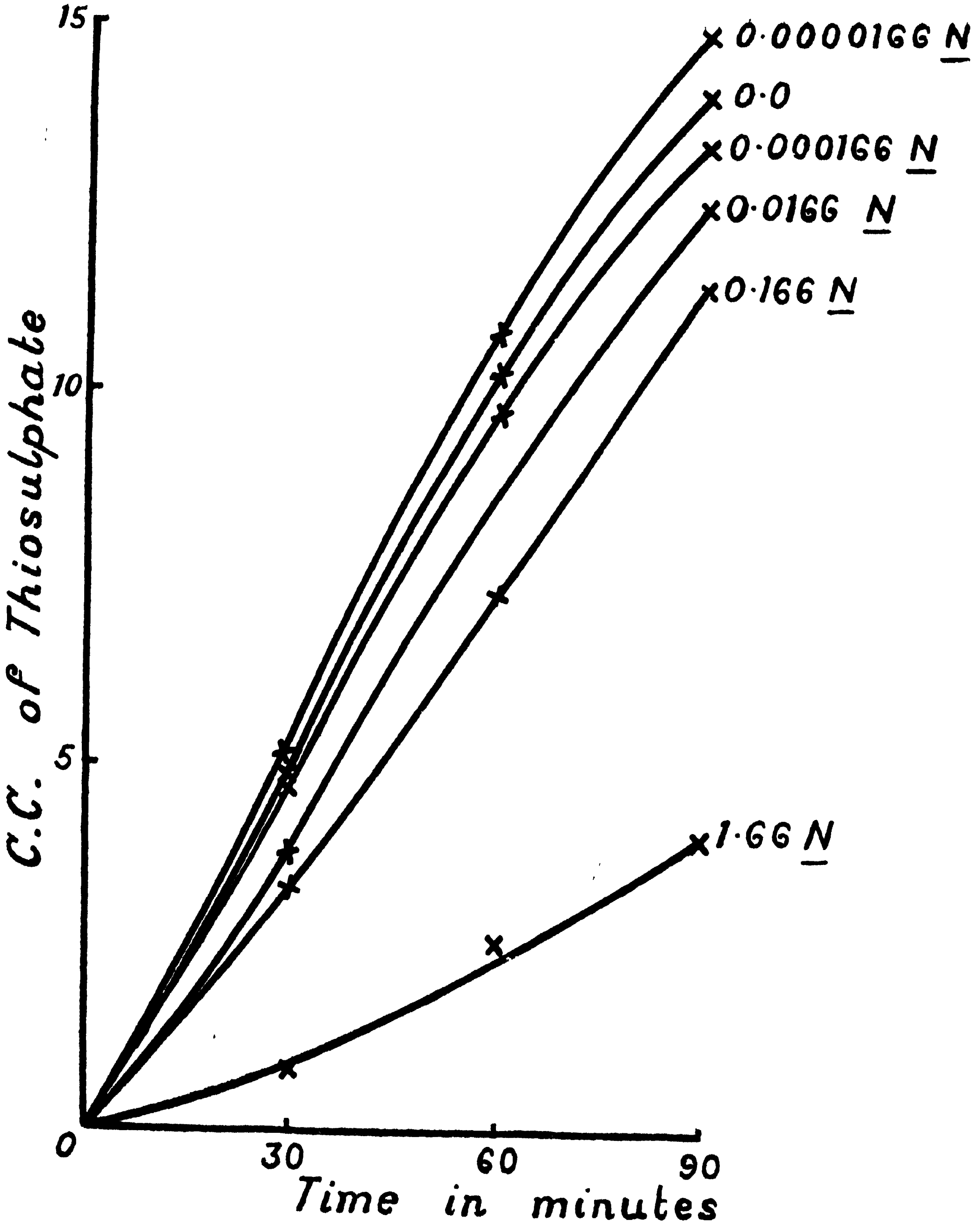
15
10
5
0
30
60
90
Time in minutes
C.C. of Thiosulphate
0·0000166 N
0·0
0·000166 N
0·0166 N
0·166 N
1·66 N

Fig. II

molecules should be greatest near the isoelectric point of the surface concerned, which has been found to be the case in hydrogen peroxide decomposition at a variety of surfaces (Wright and Rideal, *Trans. Faraday Soc.*, 1928, **24**, 530).

Influence of neutral salt concentration.—The foregoing observations suggest that the action of neutral salts on enzymes alters their electrokinetic potential, and consequently the adsorption and decomposition of neutral molecules at enzyme surfaces. This receives support from the results shown in Fig. I and also from the influence of neutral salt concentration. As will be seen from Fig. II, where the order of addition was enzyme, neutral salt, amylose, the action of sodium chloride on diastase varies with the concentration of the salt. At concentrations varying from $1{\cdot}66N$ to $0{\cdot}000166N$ it had an inhibiting action, while at $0{\cdot}000016N$ it had a slightly accelerating action. The possible explanation of the different concentration effects seems to be that the neutral salt at very low concentration reduces the charge on the enzyme particle; this does not involve any great decrease in stability, because before coagulation it must not only be discharged but also desolvated. The adsorption of electrically neutral amylose is thereby increased, thus accelerating the speed of hydrolysis. On the other hand, with increasing concentration of the salt, probably the charge on the particle is neutralised and reversed in sign, and at still higher concentrations increased in magnitude thus cutting down the power of adsorption. Though what has been outlined above seems probable, it should not be forgotten that electrolytes at very high concentrations may coagulate the enzyme particles and so decrease the active surface. It has also been observed in the case of other colloidal systems that electrolytes which at high concentrations act as discharging agents may increase the charge at low concentrations thus increasing the stability and probably the dispersion also. It is hoped that electro-kinetic potential and other physico-chemical measurements in progress will throw light on this question. It must also be mentioned that adsorption alone cannot account for the hydrolysis. The activation of water has been a matter of speculation. Recently Bancroft (*J. Physical Chem.*, 1926, **30**, 1194) has suggested that the action of neutral salts shifts the water-equilibrium, and has adduced indirect evidence in favour of this hypothesis by which L. E. Bowe has explained the beneficial action of neutral salts on acid hydrolysis. It has been suggested that at the interface, an oriented layer of molecules (Harkins, *J. Amer. Chem. Soc.*, 1925, **47**, 2083) may be largely composed of monohydrol. King and Lassieur (*Compt. rend.*, 1923, **117**, 109) have suggested that the monohydrol is a conductor of electricity while polyhydrols are not; this indicates that water in the monohydrol form is more reactive. Briggs (*Colloid Symposium monograph*, 1928, **6**, 41) finds that with alkali salts the surface conductance of cellulose increases with increasing concentration while the zeta potential falls; in the case of thallium and aluminium salts, however, the surface conductance also falls. He believes that the surface conductance is to be attributed to the monohydrols and thus thinks that the water equilibrium is favourably shifted with alkali salts.

Influence of neutral salt at various P_H ranges.—The results given in Tables I and II indicate that the neutral salt is practically without any effect at the two P_H optima, has an accelerating effect on the acid side of the optimum P_H and that inactivation is caused on the alkaline side.

TABLE I.

The optimum P_H.

P_H. ...	6·02	5·57	5·3	5·1	4·99	4·8	4·63	4·43	4·05	3·42
Activity in c.c. thiosulphate ...	4·4	5·3	6·0	5·4	6·2	5·7	5·5	4·8	4·1	0·4

The reaction mixture contained 15 c. c. of 0·58 per cent. amylose, 5 c. c. of enzyme, 5 c. c. of buffer of required P_H and 5 c. c. of either water or 0·0000166*N* sodium chloride. 1 c.c. of toluene was added as antiseptic.

TABLE II.

P_H.	Maltose in c.c. thiosulphate		
	Without neutral salt.	With neutral salt.	Change in activity.
6·02	8·9	8·1	− 0·8
5·37	9·3	9·2	− 0·1
4·99	9·3	9·4	+ 0·1
4·27	6·2	7·3	+ 1·1

Further support to the theory of diastase action outlined above is given by the observations recorded by several other workers. Höber observed that salts were without effect on the acid side of the isoelectric point of the colloidal part of the enzyme complex, and had an inhibiting action on the alkaline side. Sherman found that the isoelectric point of barley malt diastase lies near its optimum P_H. Impure invertase wanders to the anode, and after kaolin adsorption it wanders to both poles. According to Michaelis the undissociated

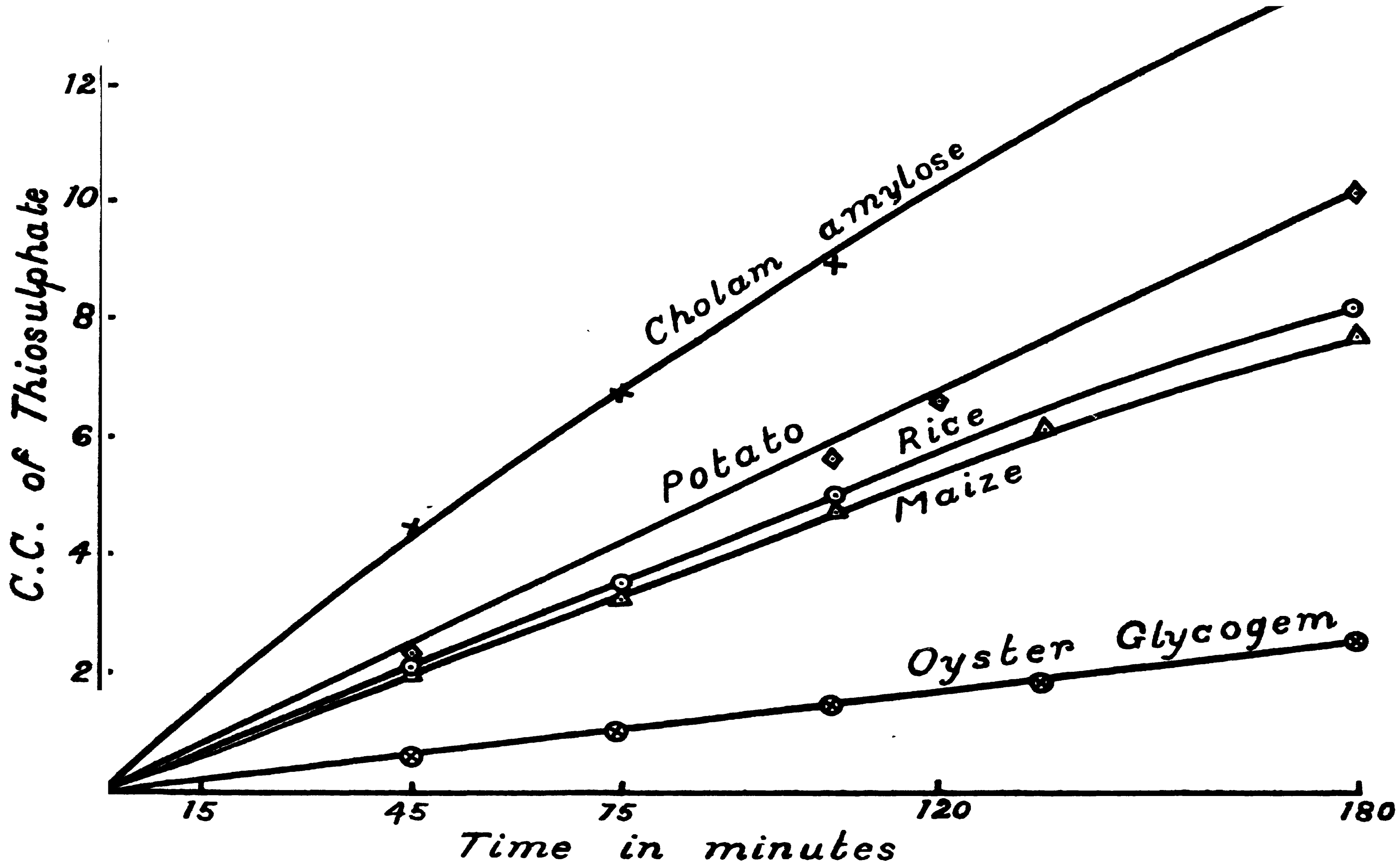
C.C. of Thiosulphate
12
10
8
6
4
2
Cholam amylose
Potato
Rice
Maize
Oyster Glycogem
15
45
75
120
180
Time in minutes

Fig. III

molecule is active in the case of invertase. It has been shown by the author (*J. Indian Inst. Sci.*, 1929, **12A**, 109) that tyrosinase acts best near its isoelectric point. Reiner has shown that frequently the end reaction in electro-dialysis is the isoelectric point of the colloid concerned; this probably explains partly the increase in activity on electro-dialysis and also the shift of the optimum P_H which frequently varies with conditions. It was noticed by the author that the optimum P_H of cumbu diastase varies with age, and a similar shift has been observed by Sherman (*J. Amer. Chem. Soc.*, 1928, **50**, 2532) on addition of salts. Hahn and Hapruder found that the optimum P_H lies in a more alkaline P_H with phosphate buffer than with acetate. Rohman (*Kolloid Z.*, 1926, **39**, 159) found that the isoelectric point with a citrate buffer lies between 4·4 and 4·6, in the case of a phosphate buffer between 5·4 and 5·7.

Divergent results have been obtained with different starches. Stone (*U. S. Dept. Agric. Bull.*, 1896, **34**, 29) found potato starch to be the most easily hydrolysed, then wheat and last maize. Sigmond (*Woch. Brau.*, 1897, **14**, 412) found a similar sequence, but according to Barnatzky (Karrer, *Polymere Kohlenhydrate*, 1925, 43), rice-starch is the most easily hydrolysed. Sherman and Baker (*J. Amer. Chem. Soc.*, 1916, **38**, 1885) noticed that amylose is more easily hydrolysed than amylopectin. Many have tried to correlate the varying amylopectin content with the varying resistance towards diastase, but according to Samec potato starch is richest in amylopectin and according to most investigators it is the most easily hydrolysed. Moreover, Sherman (*J. Amer. Chem. Soc.*, 1919, **41**, 1123) found that the different starches on washing with dilute alkali were hydrolysed at the same rate. It was found by the author that the hydrolysability of the different starches decreased with increasing ash-content. Thus cholam amylose was most easily hydrolysed, then potato starch, next rice and last maize. So there is hardly any meaning in the assumption that the different starches vary in composition. The differences may be entirely attributed to the inorganic and other impurities accompanying them and the physical characteristics of the starch. The results are given in Fig. III. Some unpublished experiments of the author also seem to support the same view. Amylose was found to be hydrolysed at the same rate as amylopectin. On another occasion amylopectin was hydrolysed more easily than amylose and yet in a third separation amylose was more rapidly hydrolysed. A systematic investigation of the question has been undertaken.

In this connection it is interesting to record the results of some experiments on the hydrolysis of amylopectin prepared by two different methods. Samec's method is well known. The other method devised by the author was based on the following considerations. Weimarn has shown that by mere grinding of substances (sometimes with the addition of an indifferent substance) at low temperatures even denatured protein can be brought back into solution. Low temperatures, especially that of liquid air confer brittleness on the substance so that it can be ground very fine, and the meal is easily peptised by water. Starch has to be gelatinised to effect a thorough disintegration of the granule before amylopectin is separated from it. For gelatinisation either heating under pressure or chemical additions have been tried and have the disadvantage of effecting chemical changes. It was interesting therefore to effect the disruption of the granule by a purely mechanical process. Applying Weimarn's idea the starch was exposed to the temperature of liquid air, ground finely in an agate mortar and the treatment repeated several times; it was then dissolved in hot water and the amylopectin separated by electro-dialysis. The results, given in Fig. IV, clearly indicate that the amylopectin prepared by the liquid air method is more easily hydrolysed, it being immaterial whether the comparison is made with or without buffer.

SUMMARY.

The order of adding the reaction mixture components has a marked influence on the activity of the enzyme; the maximum effect of the neutral salt is obtained when it is added to the enzyme before adding the substrate.

Sodium chloride at a concentration of $0{\cdot}0000166N$ has a slightly accelerating action on cholam malt diastase. At concentrations of $0{\cdot}000166N$ and above it has an inhibiting action. Sodium chloride of $0{\cdot}0000166N$ in buffered solutions has an accelerating action on the acid side of the optimum P_H, no action at the optimum P_H and a retarding effect on the alkaline side.

Experiments on the hydrolysis of different starches, and amylopectin prepared by two different methods are described.

The mechanism of diastase action has also been discussed.

The author has pleasure in expressing his indebtedness to Prof. R. V. Norris for the keen interest taken during the course of this work and the constant encouragement given.

Department of Bio-Chemistry,
Indian Institute of Science,
Bangalore.

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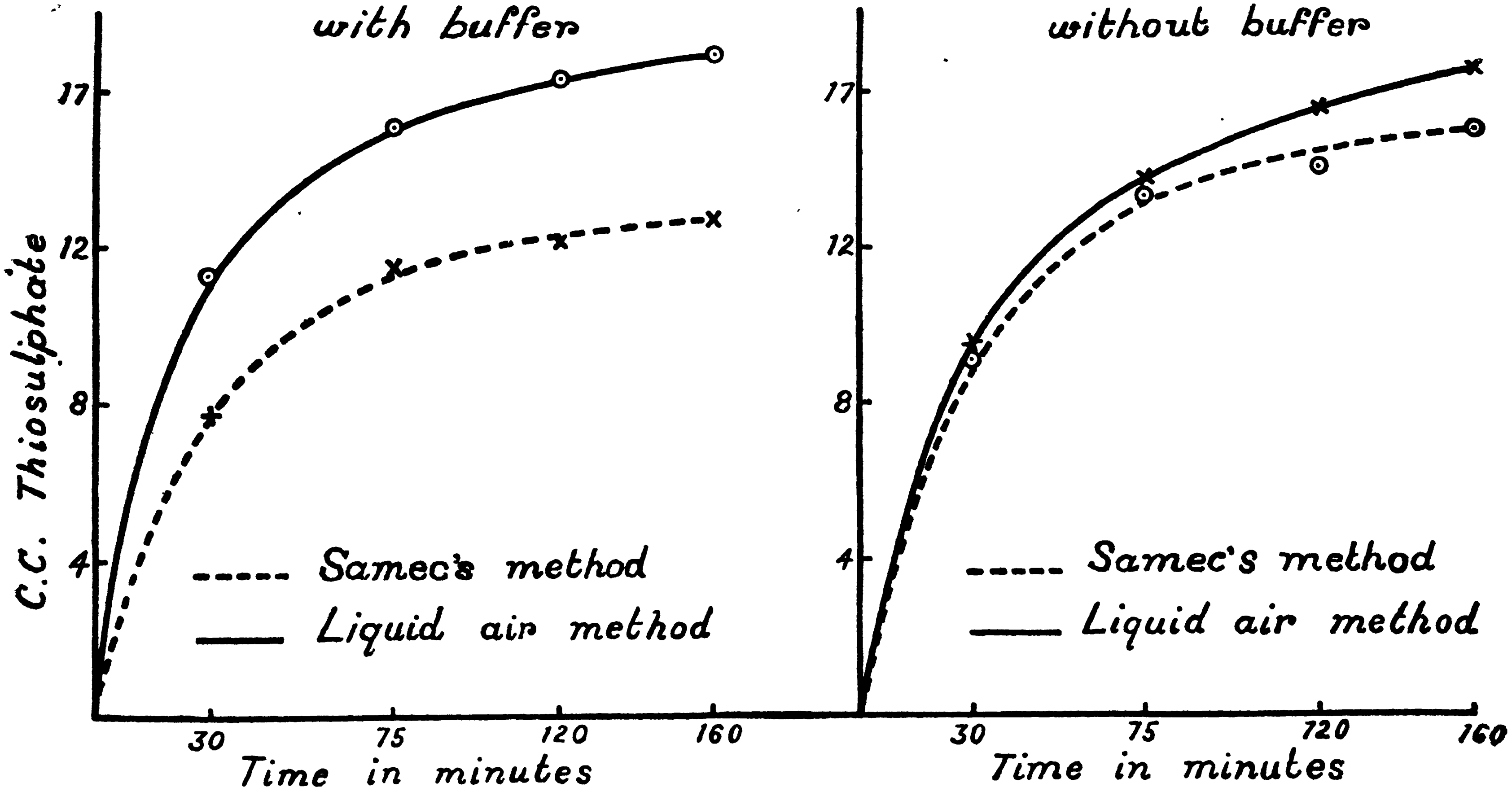
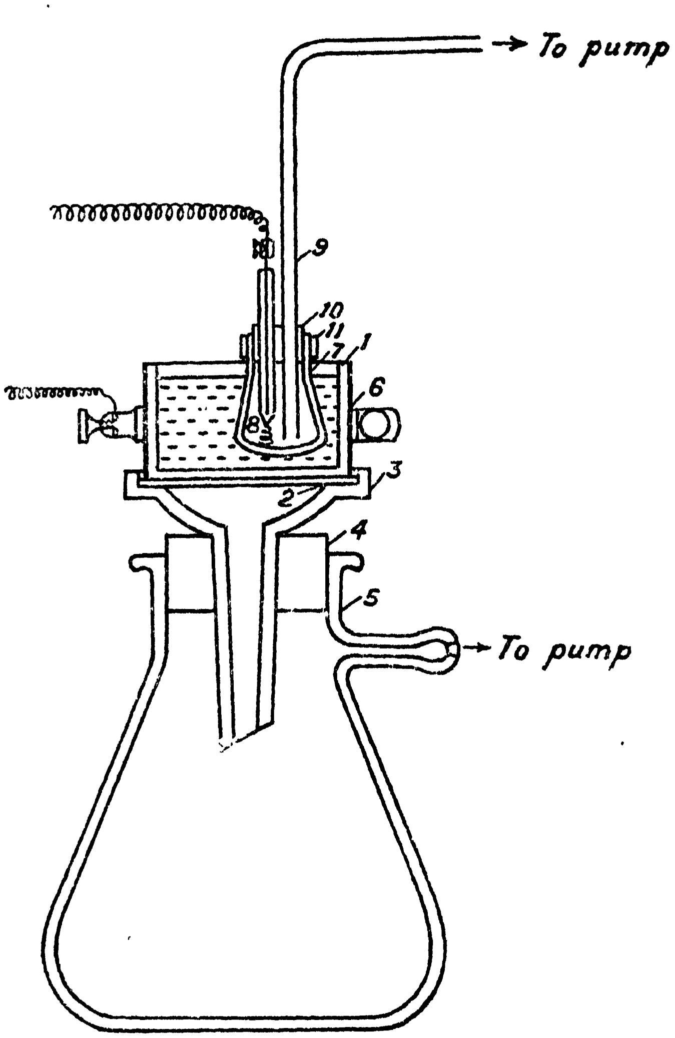

with buffer
without buffer
C.C. Thiosulphate
17
12
8
4
17
12
8
4
----- Samec's method
—— Liquid air method
----- Samec's method
—— Liquid air method
30
75
120
160
30
75
720
160
Time in minutes
Time in minutes

Fig IV



After Bechhold

1. *Cylindrical dish.*
2. *Rubber washer.*
3. *Funnel.*
4. *Rubber stopper.*
5. *Suction flask.*
6. *Brass collar nickel-plated.*
7. *Flask.*
8. *Spiral platinum electrode.*
9. *Glass tube.*
10. *Rubber stopper.*
11. *Clamp.*

Fig. I.

II.—THE NATURE OF AMYLASE. II.

By D. Narayanamurti.

Experiments on the purification of amylase by electro-dialysis and the resolution of the enzyme into two components by electro-osmosis were described in Part I (*J. Indian Inst. Sci.*, 1928, **11**A, 134). The enzyme subjected to electro-ultrafiltration has now been studied, and further evidence in favour of the two-enzyme theory follows also from ultrafiltration experiments.

ELECTRO-ULTRAFILTRATION.

This elegant and very useful process, devised by Bechhold (*Z. Elektrochem.*, 1925, **31**, 496), depends on the following principle. The collodial solution to be purified is placed on an ultrafilter from which the dissolved crystalloids are drained by suction; simultaneously an electric current removes ions and transfers water to one of the electrodes. It is thus a combination of electro-dialysis and ultrafiltration, and has the advantage of quickly removing all ionogenic and crystalloidal impurities from colloidal solutions. This is of fundamental importance when studying the action of salts on enzymes. In the case of oxidases it should be easy to settle the much discussed question whether manganese and iron, either as inorganic salts or part of the enzyme complex, are necessary for the action of these enzymes. Such a study is now being conducted by the author with tyrosinase. It is claimed that under favourable conditions the removal of electrolytes is about 180 times as rapid as by dialysis. Furthermore, bio-colloids liable to attack by micro-organisms can be quickly purified, and instead of dilution taking place concentration can be effected. Another advantage is that the process can be so conducted that the solution remains neutral or becomes acidic or alkaline; Bechhold has used it with great success for the purification of glue and gelatine, the ash after purification consisting entirely of silica and ferric oxide. The following experiments with amylase are the first of their kind with enzymes.

The Apparatus.—The electro-ultrafiltration vessels shown in Fig. I are made of special porous porcelain and resemble the ultrafiltration apparatus of Bechhold-König; they are covered with an ultrafilter membrane, and differ from ordinary ultrafiltration vessels in having an electrode of deposited platinum underneath. The deposit extends up the sides of the vessel in several places in order that electrical contact may be made by means of a tightly clamped collar.

Deposition of the membrane.—The dish is placed on a rubber washer in the funnel and 6 per cent. collodion poured to the brim, excess being poured back to the stock bottle by rotating the dish till no more drops fall. After drying in air for 5 minutes the dish is given two more similar coatings and then allowed to dry for 15 minutes before immersing in water for gelatinisation of the membrane. The flask is coated in similar manner by dipping in a beaker of collodion and applying suction for a minute.

Purification of the enzyme—The enzyme solution is placed in the dish and distilled water in the ultrafilter flask, suction being applied on both sides. The electro-ultrafiltration was effected at 30 volts, tapped by means of a potentiometric arrangement, a milliammeter in series and a voltmeter in parallel being included in the circuit to follow the course of the electro-ultrafiltration. The ammeter reading became constant (almost zero) in the course of seven hours when the current was allowed to pass for an hour more to remove the last trace of electrolyte. Neutrality disturbance was avoided by having a more powerful suction at the anode than at the cathode, which being smaller is in the flask. In some experiments acidification was avoided by reversing the direction of the current every 2 or 3 minutes.

The results obtained with cholam malt diastase, partially dialysed according to Euler, are represented in Fig. II. The solid content of the cholam diastase solution was 4·8 times that of the electro-ultrafiltered solution. The activity of the enzyme being proportional to its concentration at low concentrations the electro-ultrafiltered solution might be considered to be 4·8 times as active as the dialysed preparation. It is evident from the figure that the curves are not similar, that representing the electro-ultrafiltered solution being autocatalytic in form. Whether this has anything to do with the two-enzyme theory of diastase only future experiments can decide. It must also be mentioned that the increase in activity obtained is not as great as in electro-dialysis. Experiments are in progress to study the effect of denser membranes and the most favourable conditions are being sought.

ULTRAFILTRATION.

In the earlier communication (*loc. cit.*) evidence was adduced in favour of the theory that diastase consists of two components, one responsible for liquefaction and the other for saccharification of starch; in one series of experiments a complete separation of the liquefier from the saccharifying component was effected. Ultrafiltration experiments described below give further support to this theory.

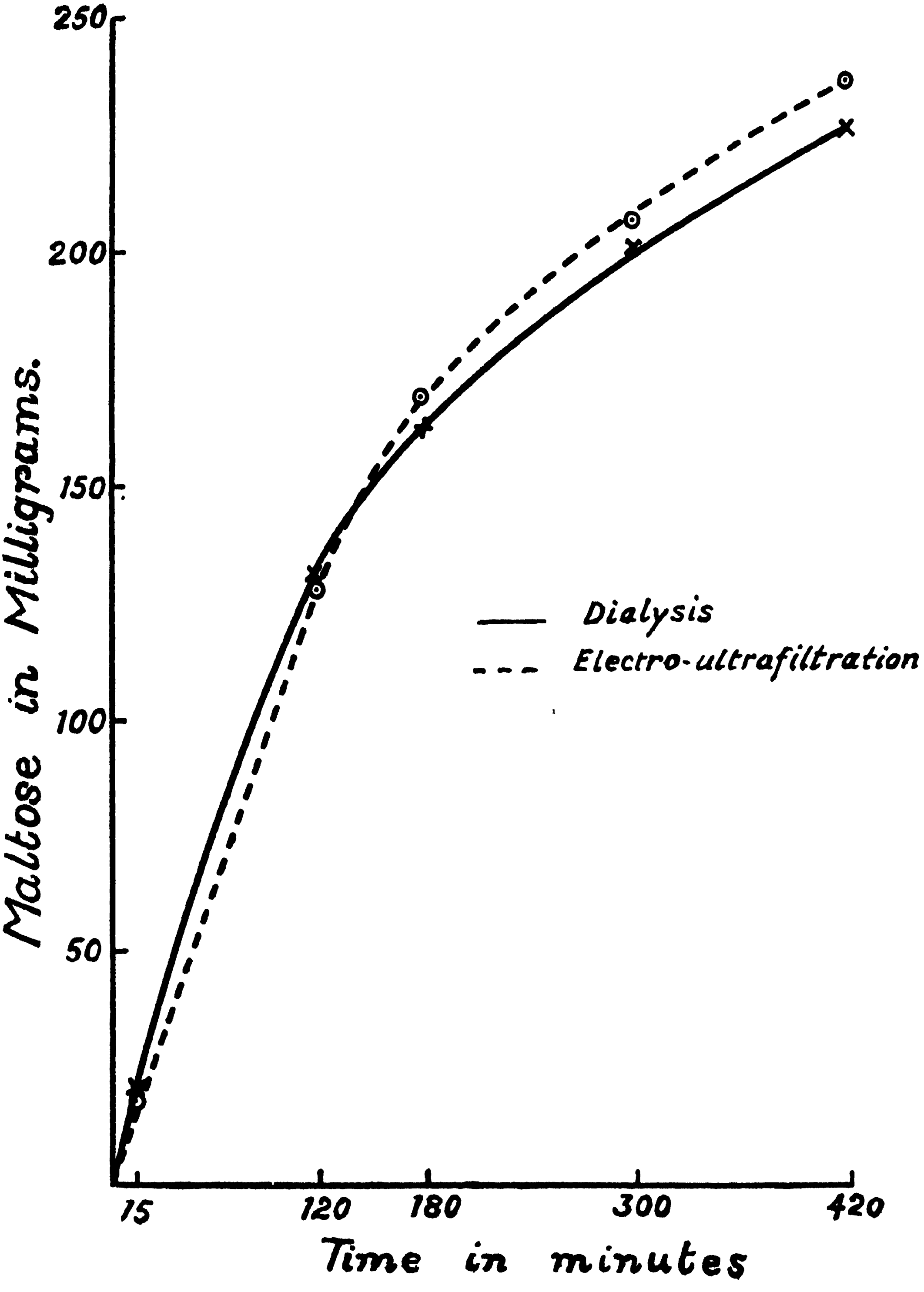
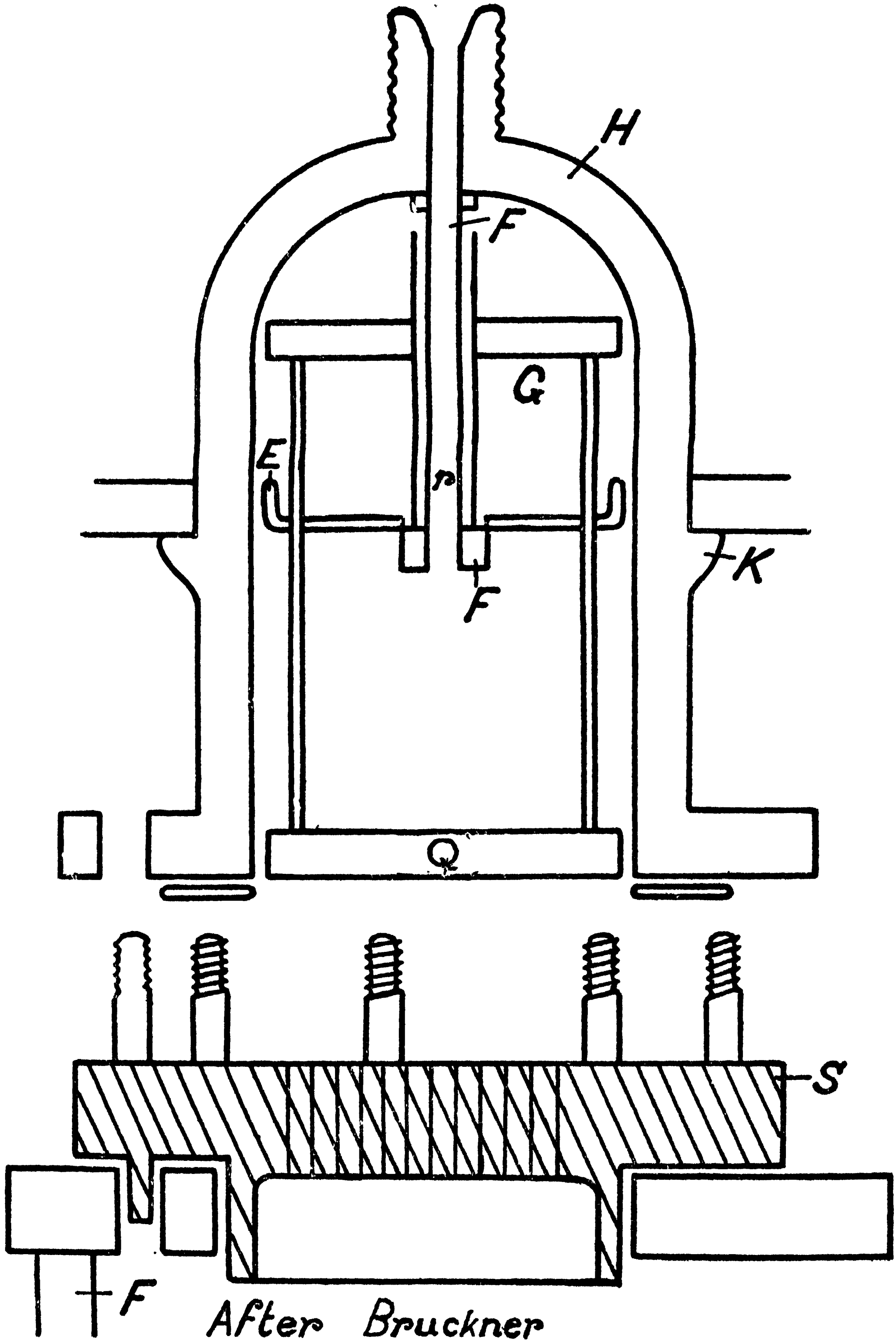

250
200
150
100
50
Maltose in Milligrams.
Dialysis
Electro-ultrafiltration
15
120
180
300
420
Time in minutes

Fig. II



After Bruckner

Fig. III

It was shown by Bechhold (*Z. physikal. Chem.*, 1908, **60**, 257) that colloids could be separated from their dispersion media by appropriate filters which he named ultrafilters, showing that by their use colloids of different dispersities could be separated from one another.

It was observed by Brückner (*Z. Ver. deut. Zucker-Ind.*, 1926, **76**, *Technischer Teil*, 837) that Zsigmondy's ultrafine filters in pressure filtration, especially at high pressures show some peculiarities. Filters which at a pressure of one atmosphere are permeable to protein become impermeable at 25 or 30 atmospheres. This could not be explained as due to a layer of protein on the filtering surface for the same phenomenon is observed when the filtration is accompanied by stirring. Brückner therefore concludes that at high pressures the pores become narrowed, the degree of change depending on the time during which the high pressure acts on the filter; further that the change is only partly reversible and that dense filters are more resistant to this change than more porous ones.

In ultrafiltration of colloidal solutions under pressure two possibilities arise. By choosing suitable filters particles of different dispersities could be separated from one another, or by using one filter, the filtrates collected at different pressures should contain the particles of different dispersities in different proportions. Moreover, in systems having an emulsoidal character, the particles which normally may be bigger than the pores of the filter might become elongated at high pressures and pass through. The results of some experiments on the ultrafiltration of diastase solutions with parchment as the filtering membrane at two different pressures are given below.

The apparatus.—The apparatus used was the one described by Brückner with slight modifications as indicated in Fig. III. It is made of brass, the parts which come into contact with the solution being tinned. It consists of the pressure dome H provided with a manometer, the sieve plate *S* about 14 mm. thick, the stirring arrangement *r* and a tripod on which the entire apparatus stands. The capacity of the dome is about 250 c.c., the upper surface of the sieve plate is smooth and three to six filter papers are placed thereon surmounted by the membrane. Nine screw bolts press the sieve plate and the dome firmly together. After filling the apparatus with compressed air, nitrogen, or carbon dioxide the valve of the manometer is closed.

The stirrer consists of a carrier *G*, attached to small tube *F* through a guide bush. It has a cross-piece *Q* moving a few millimetres above the surface of the filter. The armature *E* can be rotated by means of an electromagnet outside the dome.

Ultrafiltration of the enzyme.—B.D.H. diastase (1·5 g.) was dissolved in distilled water and made up to 250 c.c. of which 200 were used for the ultrafiltration experiments reserving the rest for comparison. Compressed air was used and samples collected at 15–20 and 40–50 kgms. The two fractions were tested for their liquefying and saccharifying powers, results being given in Figs. IV, V, VI, and VII and showing that a partial separation of the two components has been effected. The liquefier is probably of a higher dispersity (cf. *J. Indian Inst. Sci.*, 1928, **11A**, 134). Experiments are in progress to study the possibility of a complete separation of the two components by suitable choice of membranes and pressures.

SUMMARY.

Electro-ultrafiltration as a method for the purification of enzymes has been tried for the first time. Electro-ultrafiltered diastase is more active than the ordinary dialysed preparation.

The kinetics of starch-hydrolysis by the electro-ultrafiltered enzyme differs from that of the dialysed preparation. The possible significance of this to the two-enzyme theory has been indicated.

By ultrafiltration a partial separation of the two components of diastase has been effected.

Ultrafiltration experiments indicate that the liquefying component is probably of a higher dispersity than the saccharifier.

In conclusion the author desires to express his grateful thanks to Prof. R. V. Norris for the kind interest he has taken in this work.

Department of Bio-Chemistry,
Indian Institute of Science,
Bangalore.

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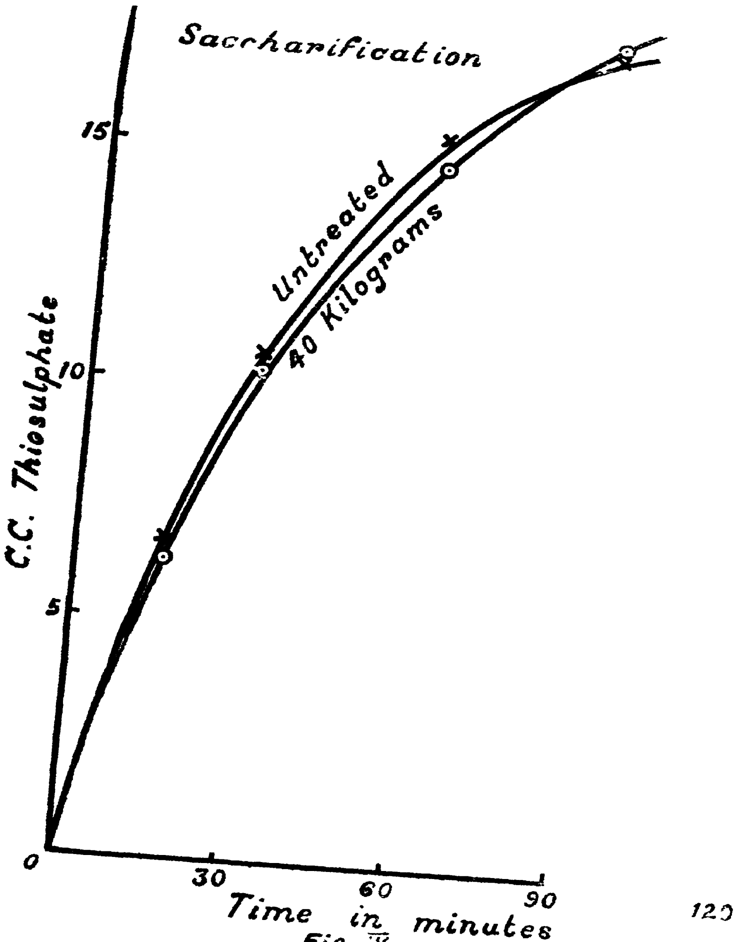
Saccharification
C.C. Thiosulphate
15
10
5
0
30
60
90
120
Time in minutes
Untreated
40 Kilograms

Fig. IV

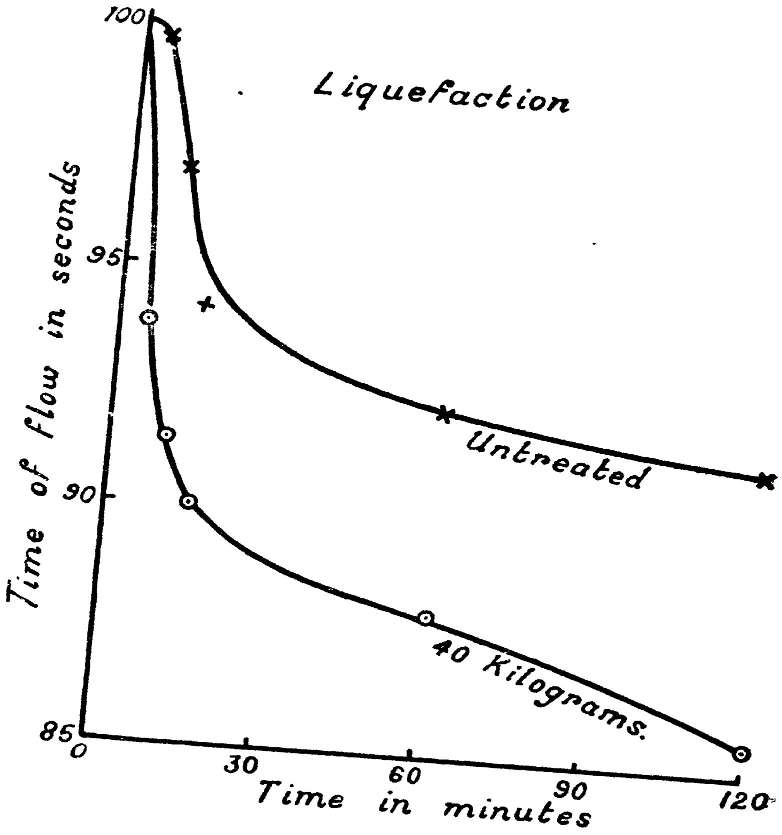
Liquefaction
Time of flow in seconds
100
95
90
85
0
30
60
90
120
Time in minutes
Untreated
40 Kilograms.

Fig. V

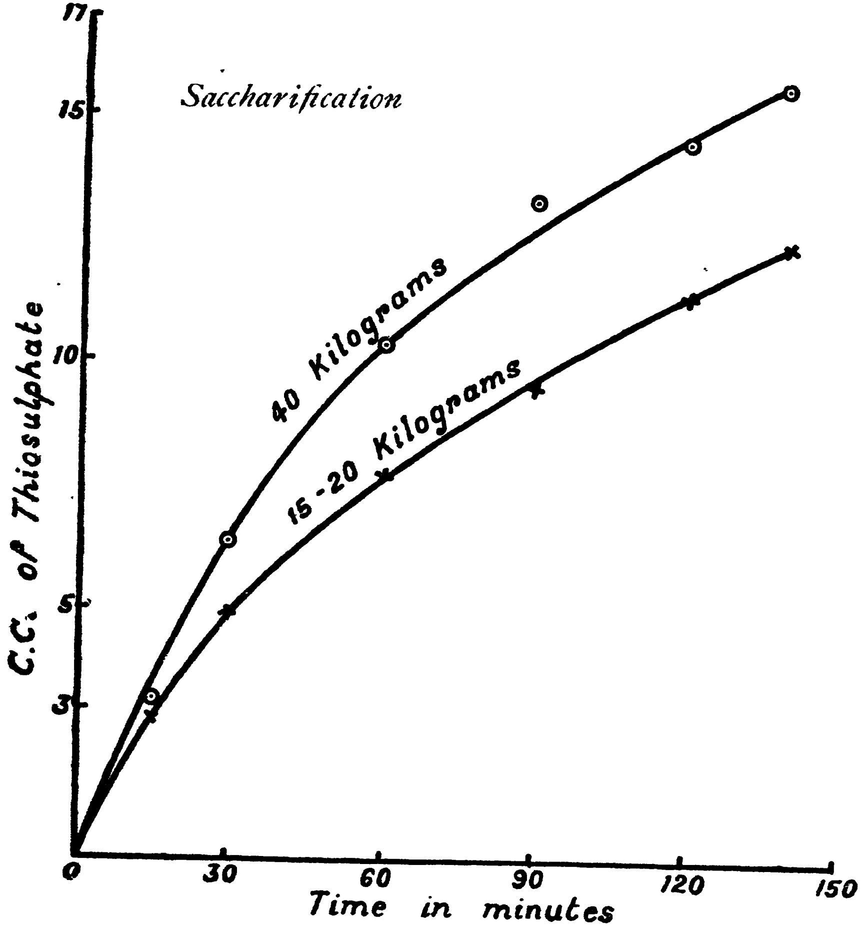

Fig. VI

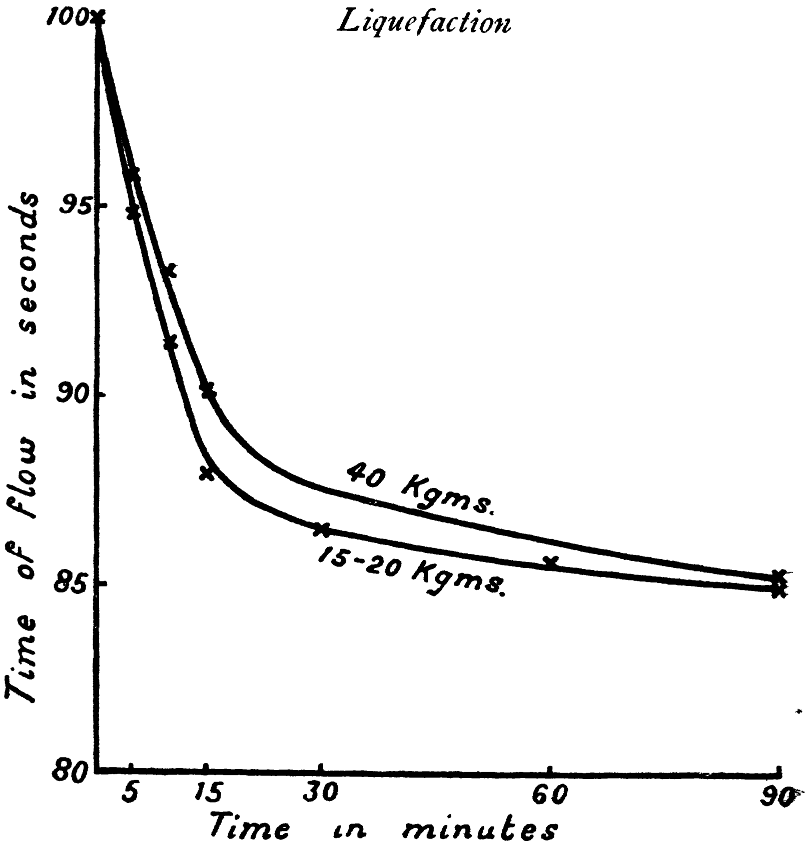

Fig. VII

I.—ACTION OF SULPHUR MONOCHLORIDE ON MERCAPTANS. PART II.[1]

Formation of Organic Trisulphides and Hexasulphides.

By P. P. Patel, I. Sengupta and G. C. Chakravarti.

The formation and structure of organic straight chain polysulphides as high as the pentasulphide have received considerable attention from several investigators and in most cases general methods have been elaborated for the preparation of such compounds. Spring and Demarteau (*Bull. Soc. Chim.*, 1889, iii, **1**, 314) obtained diethyl disulphide by the interaction of ethyl bromide or iodide and aqueous sodium polysulphides. This reaction was shown by Blanksma (*Rec. trav. chim.*, 1901, **20**, 121; *Proc. K. Akad. Wetensch., Amsterdam*, 1901, **3**, 81) to be a general one and he prepared several aliphatic and aromatic disulphides from the respective halides and sodium sulphide. He claims that tri- and tetrasulphides also can be obtained by treating the halides with sodium tri- and tetrasulphides; but the work of Thomas and Rule (*J.C.S.*, 1917, **111**, 1063) throws much doubt on the existence of alkali trisulphides themselves and in that case the organic trisulphides obtained by Blanksma have to be regarded as produced by degradation of higher polysulphides. Recently the preparation of pure anhydrous alkali tetra- and pentasulphides by Rule and Thomas (*J.C.S.*, 1914, **105**, 177, 2819) has enabled Thomas and Riding (*J.C.S.*, 1923, **123**, 3271; *ibid.*, 1924, **125**, 2214, 2460) to standardise methods for the synthesis and study of the organic pentasulphides; these authors are the first to prove by molecular weight determination in the case of the ethyl compound and by examining the tetra-iodo-derivative of the allyl compound, that these pentasulphides are definite entities and not solutions, or mixtures of the lower sulphides with sulphur.

Many workers have obtained organic polysulphides by different methods. Otto (*J. pr. Chem.*, 1888, ii, **37**, 211) claimed to have obtained phenyl tetrasulphide by the action of hydrogen sulphide on phenylsulphinic acid. Troeger and Hornung (*ibid*, 1899, ii, **60**, 133) prepared a few organic tri- and tetrasulphides by the action of sulphur dichloride and monochloride respectively on mercaptans in carbon disulphide solutions. Holmberg (*Annalen*, 1908, **359**, 81) obtained various polysulphides by the interaction of mercaptans and sulphur monochloride, sulphur dichloride, thionyl chloride, etc., but failed to obtain ethyl tetrasulphide from ethyl mercaptan and sulphur monochloride. Thomas and Riding (*loc. cit.*) also failed to obtain ethyl tetrasulphide, but Chakravarti (*J.C.S.*, 1923, **123**, 954) has shown that this can be obtained very easily by modifying Holmberg's method and he thus prepared several other organic tetrasulphides. Smythe and Aquila Forster (*J.C.S.*, 1910, **97**, 1195) have obtained benzyl di- and trisulphides by the slow action of sulphur dioxide on benzyl mercaptan, and benzyl tetrasulphide by the action of sulphur monochloride on the mercaptan in carbon tetrachloride solution.

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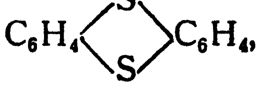
[1] Part I appeared in the *Journal of the Chemical Society*, 1923, **123**, 954.

Some polysulphides have been prepared by the action of sulphur on alkyl monosulphides (Muller, *J. pr. Chem.*, 1871, ii, **4**, 40; Klason, *ibid*, 1877, ii, **15**, 216) while Hinsberg (*Ber.*, 1910, **43**, 1874–79) states that disulphides when heated in a sealed tube to a high temperature give a mixture of mono- and trisulphides in the proportion required by the equation

$$2R_2S_2 = R_2S + R_2S_3.$$

Thomas and Riding (*loc. cit.*), however, in an extended investigation of these compounds, have found no evidence of such reactions.

Organic hexasulphides have not yet been recognised and no method is known by which they can be obtained. The only mention in the literature of a hexasulphide is by Onufrowicz (*Ber.*, 1890, **23**, 3370) who obtained an uncrystallisable solid by the action of sulphur monochloride on benzene containing a small quantity of iodine, and which on analysis corresponded to diphenyl hexasulphide; as it could not be purified, it may be a mixture of many substances and the sulphur might have attacked two or more points in the benzene nucleus. The behaviour of sulphur monochloride towards aromatic hydrocarbons in presence of the aluminium-mercury couple has already been investigated by Cohen and Skirrow (*J.C.S.*, 1899, **75**, 887) who obtained diphenylene-disulphide,



from benzene.

The present work aimed at investigating the action of sulphur monochloride on mercaptans in a suitable solvent, thus in the first instance repeating Holmberg's work in a modified way. While in progress, it was observed that, although tetrasulphides in general could be obtained by Troeger and Hornung's method in carbon disulphide, and also by Smythe and Forster's modification replacing carbon disulphide by carbon tetrachloride, the action takes an altogether different course if conducted in boiling anhydrous benzene. In experiments to be presently described, sulphur monochloride, while reacting with different mercaptans in anhydrous benzene, has given the trisulphides associated with small quantities of thick oils which in several cases were separated pure by following the method of Thomas and Riding (*loc. cit.*) applied to pentasulphides. From analysis, they appear to be the hexasulphides, and the only possibility of their being liquid pentasulphides containing sulphur in solution or pseudo-solution is precluded by the fact that most of the aromatic compounds solidify in a freezing mixture whereas dibenzyl pentasulphide prepared by Thomas and Riding (*loc. cit.*) is a liquid even at —80°. Moreover, the individuality of these purified hexasulphides was tested by separating them into two or more different fractions by suitable solvents and estimating sulphur in each. There can be therefore no doubt that these hexasulphides are definite entities.

Reactions involved in the process may be represented by the following equations :—

$$2\ R{\cdot}SH + S_2Cl_2 = (RS)_2S : S + 2\ HCl \qquad (I)$$

$$(RS)_2S : S = R\ S{\cdot}S{\cdot}SR + S \qquad (II)$$

$$2\ R{\cdot}SH + S_2Cl_2 = (RS.S)_2 + 2\ HCl \qquad (III)$$

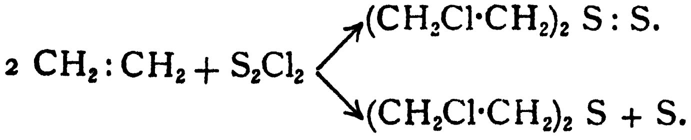
$$(RS{\cdot}S)_2 + 2S = (RS.S)_2\ S_2 \qquad (IV)$$

R being C_2H_5, $CH_3{\cdot}C_6H_4$, $C_6H_5{\cdot}CH_2$, BrC_6H_4, C_6H_5, etc.

These reactions are explained on the assumption that sulphur monochloride acts simultaneously in the forms,

$ClS{\cdot}SCl$ (*a*)
$Cl_2S{:}S$ (*b*).

in equilibrium under experimental conditions, an assumption which is not arbitrary. In studying the action of sulphur monochloride on unsaturated hydrocarbons, Guthrie (*Quart. Jour. Chem. Soc.*, 1860, **12**, 116; 1861, **13**, 134 ; *Annalen*, 1861, **119**, 91 ; 1862, **121**, 110), Pope (*J. Soc. Chem. Ind.*, 1919, **38**, 469 R), Green (*ibid*, 469 R) Conant, Hartshorn and Richardson (*J. Amer. Chem. Soc.*, 1920, **42**, 585), Gibson and Pope (*J. C. S.*, 1920, **117**, 271) and Mann, Pope and Vernon (*ibid*, 1921, **119**, 634) found that sulphur monochloride reacts with ethylene in the form (*b*) :—



$$2\ CH_2{:}CH_2 + S_2Cl_2 \begin{cases} \nearrow (CH_2Cl{\cdot}CH_2)_2\ S : S. \\ \searrow (CH_2Cl{\cdot}CH_2)_2\ S + S. \end{cases}$$

However, the recent study by Naik and his co-workers (Naik, *J. C. S.*, 1921, **119**, 379, 1231; 1922, **121**, 2592 : Naik and Bhatt, *J. Indian Chem. Soc.*, 1927, 4, 525) of sulphur monochloride acting on methylene groups, indicates attack in the form (*a*) for dithioketones; the dithio-ethers produced are so stable that they can be nitrated to tetranitro-derivatives by fuming nitric acid. Benzyl tetrasulphide obtained by Smythe and Forster (*loc. cit.*) and many tetrasulphides obtained by Chakravarti (*loc. cit.*) and other workers are remarkably stable and should more properly be represented as $(RS{\cdot}S)_2$. Thus it seems that whether sulphur monochloride reacts in one form or the other, or in both simultaneously, depends on the class of compounds with which it combines and on the experimental conditions.

In the present case sulphur monochloride in its dual capacity produces tetrasulphides as represented in equations I and III. The tetrasulphide formed in I is decomposed at the temperature of boiling benzene into the trisulphide and sulphur as represented in II. This sulphur in its nascent state combines according to IV with the stable tetrasulphide formed in III to yield the hexasulphide. Such a conversion of lower sulphides into polysulphides by the absorption of sulphur is not unusual (Cf. Muller, *loc. cit.*; Klason, *loc cit.*). The formation of ββ-dichloro-diethyl tri- and pentasulphides from sulphur monochloride and ethylene observed by Conant, Hartshorn and Richardson (*loc. cit.*), also must be due to action of this type.

Moreover, the above explanation is established by the larger quantities of trisulphides obtained, as required by the equations, and also by the detection of di-β-naphthyl tetrasulphide formed in the case of β-naphthyl mercaptan, when the absorption of sulphur according to equation IV is very slow. It is interesting to note in this connection that Thomas and Riding (*loc. cit.*) could not find any appreciable change in a mixture of β-naphthyl halide and anhydrous potassium pentasulphide, even after more than three months. Probably such highly retarded action is characteristic of the naphthalene ring.

The only general and suitable method known for preparing open chain organic trisulphides is the one by Troeger and Hornung (*loc. cit.*), in which sulphur dichloride acts on the mercaptan thus

$$2R{\cdot}SH + SCl_2 = RS{\cdot}S{\cdot}SR + 2HCl;$$

but general opinion does not altogether favour the individuality of sulphur dichloride, as the following passage from Fritz Ephraim (*Inorganic Chemistry*, 1926, page 499) indicates.

"A chlorination product of the formula SCl_2 intermediate between SCl_4 and S_2Cl_2 was formerly supposed to exist, because liquids of that composition had a much browner colour than S_2Cl_2 and SCl_4. If, however, S_2Cl_2 is gradually saturated with chlorine, the solutions formed have properties which change continuously with variation in their chlorine content; when represented graphically, they show no break or transition point at the composition SCl_2. Thus the m.p. curve only shows maxima at the compositions SCl_4 and S_2Cl_2 and the chlorine content of the vapour rises quite regularly with increase in the chlorine content of the liquid."

Holmberg (*loc. cit.*), however, states that the compounds described as trisulphides by Troeger and Hornung are possibly equivalent mixtures of di- and tetrasulphides, for an undistilled portion of the oil, on analysis, gave the results for the trisulphide; but this is untenable, because these authors give for the trisulphides sharp melting points closely approximating to those of the trisulphides obtained in the present case. A second convenient method for preparing trisulphides is already mentioned, and appears to be the first observation in which sulphur monochloride reacting with mercaptans can form trisulphides in general. A third and perhaps the most convenient method for preparing organic trisulphides was discovered while acting with excess of sulphur on various copper mercaptides; preparation of organic trisulphides by this method also forms a part of the present work.

The method is again a modification of Holmberg's method in which he heated sodium and potassium mercaptides with sulphur, obtaining disulphides. If, however, a dry copper mercaptide is heated for 9 to 10 hours in boiling benzene, with flowers of sulphur slightly in excess, black precipitates of copper sulphide settle, while the filtered solution gives the trisulphide in good yield. The process appears to be quite simple; the mercaptide loses copper which combines with sulphur to form copper sulphide, while the radicles which otherwise would have combined to yield the disulphide, take up an atom of sulphur from its excess and form the trisulphide thus:

$$(RS)_2Cu + 2S = RS{\cdot}S{\cdot}SR + CuS.$$

This observation is important and interesting as offering strong evidence for the existence of radicles containing monovalent sulphur atoms. The work of Lecher (*Ber.*, 1915, **48**, 524; 1920, **53**, 577) may be recalled in this connection. Lecher's assumption is based on the indirect evidence that certain sulphides when heated show colorations differing from those of the ordinary solutions of the sulphides, whereas in the present instance the formation of the trisulphides cannot be explained on any other basis than that the free monovalent sulphur radicles take up molecular sulphur thus:

$$(RS)_2Cu + S = 2\,(RS—) + CuS;\ 2\,(RS—) + S = RS{\cdot}S{\cdot}SR.$$

That the trisulphide is not the product of sulphur absorption by the disulphide, if formed, was shown by an experiment with sulphur and a disulphide, all other conditions remaining the same; after the notified period the disulphide was recovered unchanged. The only alternative explanation of trisulphide formation through decomposition of the disulphide into an equivalent mixture of mono- and trisulphides, as observed by Hinsberg (*loc. cit.*), does not hold because in no case was the monosulphide obtained.

EXPERIMENTAL.

Sulphur monochloride was purified by distillation in absence of moisture from a mixture of sulphur flowers and highly absorbent charcoal.

Interaction of p-*Tolyl Mercaptan and Sulphur Monochloride: Formation of Ditolyl Trisulphide.*

Sulphur monochloride (3 g.) in anhydrous benzene (50 c.c.) was added to *p*-tolyl mercaptan (5·5 g.) also dissolved in about 50 c.c. of benzene and left under a condenser with a guard tube. A vigorous reaction ensued with evolution of hydrogen chloride, and when it had subsided, the mixture was heated under reflux for about half an hour by which time the evolution of hydrogen chloride stopped. The benzene was distilled and the oily residue subjected to steam distillation which removed excess of mercaptan and also the disulphide. The semi-solid mass obtained on cooling was separated from water and extracted repeatedly with hot glacial acetic acid which on cooling deposited an oil followed gradually by crystals. The decanted liquid gave crystals and the first precipitate of oil on standing overnight set to a pasty semi-solid mass, which also gave crystals on a porous tile. Both products were recrystallised twice from acetic acid, absolute alcohol also being applicable. Ditolyl trisulphide formed minute colourless plates melting at 82–83° ; Troeger and Hornung (*loc. cit.*) gave 76–77°, while Holmberg, who prepared it by the action of thionyl aniline on *p*-tolyl mercaptan, found 81–82° (Found: C, 60·01; H, 5·75; S, 34·96. $C_{14}H_{14}S_3$ requires C, 60·43; H, 5·04; S, 34·53 per cent.).

Ditolyl trisulphide is extremely soluble in benzene, ether, chloroform and ligroin; it is also soluble in hot alcohol. Attempts to obtain double compounds with various metallic halides were fruitless.

Ditolyl Hexasulphide.—After repeated extraction with acetic acid the semi-solid mass was further extracted five times with boiling absolute alcohol to remove lower polysulphides, when a homogenous yellowish oil was obtained. This oil (about 1 g.) was mixed with light petroleum (150 c.c., b.p. 40–60°) and kept in a closed vessel for a week, to precipitate free sulphur as described by Thomas and Riding with pentasulphides. The solution was then filtered from a small quantity of precipitated sulphur and the solvent removed, the residual oil being kept in an evacuated vessel over phosphoric oxide for 4–5 days and then analysed.

When estimating sulphur by Carius' method in compounds containing a very high percentage, low results are generally obtained due to the formation of sulphonic acids, the nitric acid alone being incapable of oxidising all sulphur to sulphuric acid; hence the fusion method is usually adopted. If, however, a few drops of bromine are added to the nitric acid, equally satisfactory results are obtained and much time is saved; all sulphur estimations of higher polysulphides in the present work were made by this method (Found: S, 50·4. $C_{14}H_{14}S_6$ requires S, 51·0 per cent.). The oil was then separated into two fractions by extractions with hot alcohol, and the higher fraction purified as above (Found: S, 50·8 per cent.).

Di-*p*-tolyl hexasulphide is a thick, yellowish brown, mobile oil having the faint but persistent disagreeable odour characteristic of polysulphides. It is soluble in cold petrol, but very sparingly in boiling absolute alcohol. When shaken in petrol solution with electrolytic copper or mercury, it readily begins to form black precipitates of metallic sulphides. With piperidine it gives a reddish-brown coloration which disappears on acidification, indicating an additive compound of this base. A sample of the ditolyl hexasulphide after six months seemed to contain a solid lower sulphide as a result of decomposition.

Interaction of Copper p-*Tolyl Mercaptide with Sulphur: Formation of Ditolyl Trisulphide.*

The powdered mercaptide (3 g.) was suspended in dry benzene (50 c.c.) and boiled under reflux for 9 to 10 hours with excess of pure powdered sulphur (0·7 g.). The black copper sulphide which slowly separated was filtered and the benzene removed by distillation; the thick yellow oil remaining was extracted with hot alcohol which on cooling deposited colourless plates of ditolyl trisulphide melting at 82–83° after recrystallisation from alcohol. It is identical with the trisulphide obtained from *p*-tolyl mercaptan and sulphur monochloride, and was not accompanied by the monosulphide.

Interaction of p-*Bromophenyl Mercaptan and Sulphur Monochloride: Formation of* pp'-*Dibromodiphenyl Trisulphide.*

p-Bromophenyl mercaptan (7·5 g.) dissolved in 50 c.c. of dry benzene was treated with 20 c.c. of dry benzene containing sulphur monochloride (2·5 g.). The reaction proceeded as in the case of *p*-tolyl mercaptan with evolution of hydrogen chloride the mixture being then heated under reflux and well protected from moisture for about an hour to complete the reaction. Benzene was distilled and the crude oily product extracted six or seven times with warm alcohol. The concentrated extracts on cooling gave yellow crystals which on recrystallisation from alcohol formed pale yellow prismatic plates, m.p. 69°. (Found: S, 24·75; Br, 38·32; $C_{12} H_8 Br_2 S_3$ requires S, 23·53; Br, 39·21 per cent.).

pp'-*Dibromo-diphenyl Trisulphide* does not seem to have been described; it is soluble in all ordinary solvents and can be best crystallised from petrol (b. p. 40–60°).

pp'-*Dibromo-diphenyl Hexasulphide.*—The residual oil after extraction in the previous experiment was extracted again two or three times, dried and kept dissolved in petrol in a closed vessel for a week when the pure hexasulphide separated as in the previous case. (Found: S, 39·13. $C_{12}H_8Br_2 S_6$ requires S, 38·1 per cent.). It is slightly deeper in colour than the *p*-tolyl compound and solidifies on cooling in ice, being a deep brown heavy oil at the ordinary temperature.

Interaction of p-*Iodophenyl Mercaptan and Sulphur Monochloride: Formation of* pp'-*Diiododiphenyl Trisulphide.*

Iodophenyl mercaptan (4·7 g.) in 50 c.c. of dry benzene was added to 14 c.c. of a 10 per cent solution of sulphur monochloride in benzene and the reaction carried out exactly as before, the solution becoming much coloured, probably on account of the liberation of iodine. The pasty mass left on evaporation of benzene was extracted with alcohol from which the *pp'*-diiododiphenyl trisulphide was obtained by fractional crystallisation; it is very unstable and becomes brown owing to separation of iodine. The freshly prepared substance, after repeated crystallisation from alcohol, forms yellow leaflets, m.p. 91° (Found: S, 19·12; I, 49·81. $C_{12} H_8 I_2 S_3$ requires S, 19·12; I, 50·65 per cent.). pp'-*Diiododiphenyl trisulphide* also has not been described. The hexasulphide is naturally expected to be very unstable and could not be obtained pure.

Interaction of Benzyl Mercaptan and Sulphur Monochloride: Formation of Dibenzyl Trisulphide.

Benzyl mercaptan (6 g.) diluted with dry benzene (30 c.c) was treated under reflux with sulphur monochloride (2·8 g.) diluted with 20 c.c. of dry benzene. When evolution of hydrogen chloride had ceased, benzene was distilled and the residual oil extracted with small quantities of alcohol which on concentration and cooling in ice deposited oily drops. These, when rubbed with alcohol, gave a solid forming minute white plates, m.p. 47–48°, on crystallisation from alcohol (Found: S, 34·42. $C_{14}H_{14}S_3$ requires S, 34·53 per cent.).

Dibenzyl trisulphide has been obtained by Smythe and Forster (*loc. cit.*) by the action of sulphur dioxide on benzyl mercaptan; they give 49° as the m.p.

Dibenzyl Hexasulphide.—The oil, after removing the trisulphide, was further extracted with hot alcohol, dried and dissolved in a large volume of petrol as before. After about a week the solvent was distilled and the oil thoroughly dried in vacuum over phosphoric oxide (Found: S, 51·77. $C_{14}H_{14}S_6$ requires S, 51·00 per cent.)

Dibenzyl hexasulphide is a faint yellow, highly refractive oil and is more stable than *p*-tolyl compound; it solidifies in a freezing mixture although dibenzyl pentasulphide obtained by Thomas and Riding (*loc. cit.*) does not solidify at —80°. Dibenzyl hexasulphide also gives with piperidine a coloration which is destroyed by acid.

Interaction of Copper Benzyl Mercaptide and Sulphur: Formation of Dibenzyl Trisulphide.

By heating together sulphur (0·5 g.) and copper benzyl mercaptide (2·3 g.) in 60 c.c. of anhydrous benzene under reflux for ten to eleven hours, an oily mass was obtained, which on cooling solidified and formed white plates, m. p. 48°, when recrystallised from alcohol. It is identical with the specimen from sulphur monochloride and benzyl mercaptan, and is not accompanied by monosulphide.

Reaction between Phenyl Mercaptan and Sulphur Monochloride.

Phenyl mercaptan (10 g.) diluted with 30 c.c. of dry benzene was treated with sulphur monochloride (3 g.) dissolved in 20 c.c. of dry benzene; when evolution of hydrogen chloride had ceased, benzene was removed and the product steam distilled. Half of the residual oil, freed from sulphur, was separated into two fractions (1) soluble in alcohol, (2) insoluble in alcohol, by repeated fractional precipitation (1. Found: S, 43·3. $C_{12}H_{10}S_3$ requires S, 38·4 : per cent. 2. Found, S, 52·0. $C_{12}H_{10}S_6$ requires S, 55·5 per cent.).

Thus the ordinary method of fractional precipitation did not succeed in this case, each sulphide appearing to be contaminated with some of the other. The second half of the residual oil was therefore distilled under 30 mm. pressure, most of the distillate being collected at 240–260° and leaving a mass resembling pasty sulphur. The fluid distillate contained a solid which was filtered at the pump and crystallised from alcohol, when it melted at 60° and was found to be *p*-diphenyl disulphide. The liquid distillate appeared to be the trisulphide. It can be said that a higher sulphide was formed during the reaction, but on distillation decomposed to the lower sulphides including the disulphide (cf. Thomas and Riding, *loc. cit.*). This reaction is the only one in the aromatic series giving rise to the tri- and hexasulphides, both of which are liquids; hence the failure in complete separation and purification of the two.

Reaction between β-Naphthyl Mercaptan and Sulphur Monochloride: Formation of ββ'-Dinaphthyl Trisulphide.

The reaction of β-naphthyl mercaptan, (7 g.) and sulphur monochloride (3 g.) was conducted in benzene under the conditions previously described. Benzene was removed, and after steam distillation the oily product extracted several times with alcohol. On concentrating and cooling the collected extracts, the white flocculent solid was recrystallised from absolute alcohol and separated into two fractions, the less soluble being again crystallised and melting at 99°. This is the ββ'-dinaphthyl tetrasulphide of Troeger and Hornung (*loc. cit.*). The second fraction on recrystallisation from alcohol gave m.p. 107–108° (Found: S, 27·68. $C_{20}H_{14}S_3$ requires S, 27·43 per cent.). In this case the sulphides being solids, separation was extremely difficult, and a sample of the hexasulphide suitable for analysis could not be obtained.

Copper β-Naphthyl Mercaptide and Sulphur.—The reaction took place as in the case of *p*-tolyl mercaptide and the product was ββ'-dinaphthyl trisulphide melting at 107–108°.

Di-ββ'-naphthyl Disulphide and Sulphur.—This experiment was designed to show whether the trisulphide is formed on absorption of sulphur by the disulphide (see introduction). Di-ββ'-naphthyl disulphide (2·5 g.), m.p. 139°, and 50 c.c. of dry benzene containing 1 gram (4 atoms) of sulphur were heated under reflux for 10 to 12 hours, almost all the disulphide being recovered unchanged.

Interaction of Ethyl Mercaptan and Sulphur Monochloride. Formation of Diethyl Trisulphide.

Ethyl mercaptan (12·4 g.) was mixed with anhydrous benzene (50 c.c.), cooled with ice water and a well cooled solution of sulphur monochloride (13·5 g.) in dry benzene gradually added with shaking. When the evolution of hydrogen chloride ceased after heating the mixture finally on water bath, the solvent was distilled and the oily liquid, with a strong odour of garlic, steam distilled. Unchanged mercaptan and the disulphide came over rapidly; the first portion of the distillate containing these substances was rejected and the oil which volatilized extremely slowly with steam was collected with ether; after drying and removing the solvent a clear reddish brown oil remained. This was freed from the last traces of ether over phosphoric oxide in an evacuated desiccator (Found: S, 62·5. $C_4H_{10}S_3$ requires S, 62·34 per cent.).

Diethyl Hexasulphide.—The brown residue from steam distillation was again subjected to a rapid current of steam for one full day, yielding a few oily drops, the residue being treated with ether which left pasty sulphur. The solution having been dried and evaporated, the hexasulphide was dissolved in a large volume of petrol and kept for a week in a closed vessel; the petrol was filtered and distilled, leaving a reddish brown oil of penetrating odour. (Found: S, 76·7. $C_4H_{10}S_6$ requires S, 76·8 per cent.). Diethyl hexasulphide slowly decomposes on prolonged steam distillation or long standing (6 months).

Propyl Mercaptan and Sulphur Monochloride: Formation of Dipropyl Trisulphide.

The reaction was conducted as in preparing the ethyl compound; after distillation in steam and fractionation, a clear yellowish mobile liquid was obtained (Found: S, 52·92. $C_6H_{14}S_3$ requires S, 52·74 per cent.) The residue was found chiefly to consist of sulphur showing that the hexasulphide was decomposed.

n-*Butyl Mercaptan and Sulphur Monochloride: Formation of Di-*n-*butyl Trisulphide.*

The reaction was conducted as before, yielding a clear yellow oil (Found: S, 45·23. $C_8H_{18}S_3$ requires S, 45·71 per cent.). The residue in the flask was further steam-distilled for some time and extracted with ether, the dry oil from which was dissolved in petrol; after several days, the filtered solution was evaporated and the pure oil analysed (Found: S, 55·83. $C_8H_{18}S_4$ and $C_8H_{18}S_6$ require S, 52·5 and 62·3 per cent., respectively). It is therefore probably a mixture of the tetra- and hexasulphides; on standing for three days solid sulphur appeared.

SUMMARY.

The organic open chain hexasulphides are a distinct class of compounds which can be obtained conveniently by the action of sulphur monochloride on mercaptans, trisulphides in considerable quantity being simultaneously produced.

Sulphur dichloride of doubtful composition thus need not be used for preparing the trisulphides.

Another most convenient method for the synthesis of these trisulphides is the action of sulphur on copper mercaptides.

The last observation is strong evidence in favour of the existence free radicles containing monovalent sulphur.

Departments of Organic Chemistry,
University College of Science & Technology,
Calcutta,
and
Indian Institute of Science,
Bangalore.

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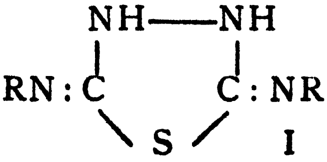
II.—ACTION OF SULPHUR MONOCHLORIDE ON MERCAPTANS. PART III.

OXIDATION OF UNSYMMETRICALLY SUBSTITUTED HYDRAZODITHIODICARBONAMIDES TO THIODIAZOLES.

By P. P. Patel and G. C. Chakravarti.

Having discussed in the previous communication, the action of sulphur monochloride on mercaptans, it seemed interesting to ascertain its effect on 'potential' mercaptans. Chakravarti (*J. C. S.*, 1923, **123**, 964) stated that sulphur monochloride is capable of distinguishing real mercaptans from potential mercaptans, thus attributing to it a property resembling that ascribed to chloropicrin by Rây and Das (*J. C. S.*, 1922, **121**, 323). Chakravarti's view was based on the partial or complete separation of sulphur when potential mercaptans react with sulphur monochloride, no separation occurring in the case of real mercaptans; he acted upon potential mercaptans, e.g., thiobenzoic acid and thiocarbanilide with sulphur monochloride and in some cases identified the products.

A typical class of potential mercaptans was chosen, for besides the confirmation of the rule observed by Chakravarti, an interesting condensation giving rise to more definite products was anticipated. It is well known that 4-substituted thiosemicarbazides of the general formula, $NHR{\cdot}CS{\cdot}NH{\cdot}NH_2$, like thiocarbamides, in their aqueous or alcoholic alkaline solutions react in the tautomeric form, $NHR{\cdot}C(SH):N{\cdot}NH_2$, or $NR:C(SH){\cdot}NH{\cdot}NH_2$, and for this the work of Guha and Sen (*J. Indian Chem. Soc.* 1927, **4**, 43) bears ample evidence. When, therefore, sulphur monochloride is allowed to react, at low temperatures, with such tautomeric forms of 4-substituted thiosemicarbazides condensation takes place and generally thiodiazoles (I) are formed along with small quantities of the corresponding hydrazodithiodicarbonamide derivatives (II):

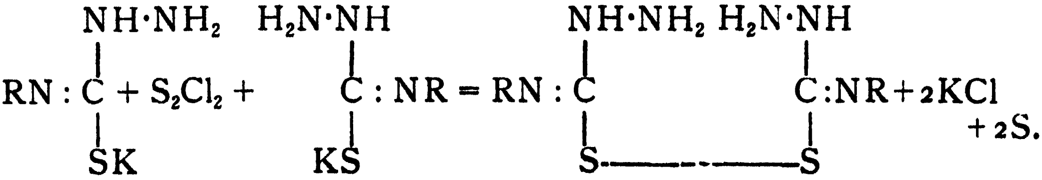


I

$NHR{\cdot}CS{\cdot}NH{\cdot}NH{\cdot}CS{\cdot}NHR.$

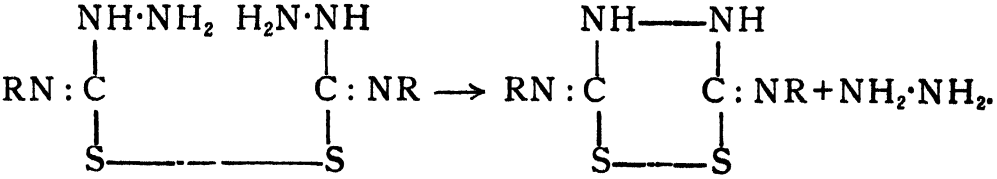
II

With respect to the mechanism of condensation, it is suggested that the first stage consists in the union of two molecules of the potassium salt of the thiosemicarbazide at the mercaptanic groups, with liberation of potassium chloride and formation of an intermediate disulphide (not isolated) thus :—



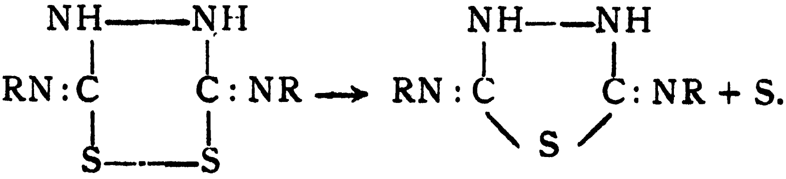
(Intermediate disulphide.)

The disulphide then, under the conditions suitable for ring closure, is converted into another intermediate compound (a dithio-ring structure) with elimination of hydrazine as follows :—



(Dithio-ring compound.)

The dithio-ring compound, like disulphides with adjacent double bonds, decomposes with separation of sulphur (Cf. From, Layer and Ners, *Annalen*, 1923, **433**, 1) to yield the thiodiazole (I) thus :—



The formation of the hydrazodithiodicarbonamide derivative (II) follows the partial decomposition of the thiosemicarbazide into hydrazine and mustard oil. While preparing the potassium derivative of the thiosemicarbazide by adding the latter to the calculated quantity of absolute alcoholic potash, a certain amount of water is formed as under :—

$$NR{:}\ C(SH){\cdot}NH{\cdot}NH_2 + KOH = NR{:}\ C(SK){\cdot}NH{\cdot}NH_2 + H_2O.$$

The water thus formed reacts partially with sulphur monochloride used in the reaction and produces hydrogen chloride, which even at ordinary temperature can decompose such thiosemicarbazides into hydrazine and the corresponding mustard oil, the latter uniting with unchanged thiosemicarbazide to produce (II) :—

$$NR{:}\,C{:}\,S + NH_2{\cdot}NH{\cdot}CS{\cdot}NHR = NHR{\cdot}CS{\cdot}NH{\cdot}NH{\cdot}CS{\cdot}NHR.$$

We have substantial evidences in support of the above mechanism. Firstly, the formation of mustard oil has been observed, and secondly, the yield of II varies directly with the temperature of the reaction, within certain limitations. In a freezing mixture of ice and salt, the formation of II was altogether checked, the only product being I, while at room-temperature, II was the only product. Moreover, in every experiment at low temperatures, the mustard oil was found to have been formed. These observations show that when the temperature is sufficiently low, though slight decomposition into the mustard oil and hydrazine takes place, reaction of the mustard oil with the thiosemicarbazide does not occur, but that at comparatively higher temperatures decomposition proceeds to the formation of II.

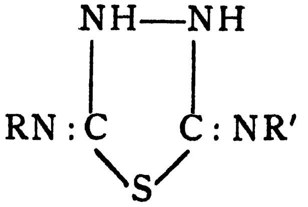
That hydrochloric acid is responsible for changing the thiosemicarbazide to the hydrazodithiodicarbonamide derivative was confirmed by an independent experiment. When a small quantity of 4-phenylthiosemicarbazide was heated for a short time with dilute alcoholic hydrochloric acid solution, sparingly soluble *Sym*-diphenylhydrazodithiodicarbonamide was precipitated. In this connection it should be pointed out that the conclusion of Pulvermacher (*Ber.*, 1893, **26**, 2812 ; *ibid*, 1894, **27**, 613) that phenylthiocarbamide is formed by the action of hydrogen chloride on 4-phenylthiosemicarbazide, seems doubtful.

The action of sulphur monochloride on 4-phenyl, 4-*p*-tolyl- and 4-*o*-tolylthiosemicarbazides has been studied and the products described. With 4-phenylthiosemicarbazide under different conditions interesting results were not obtained. On slowly adding the agent in chloroform to a solution of 4-phenylthiosemicarbazide in the same solvent probably an unstable double compound was formed, giving sulphur and phenyl thiosemicarbazide hydrochloride on treatment with alcohol or water. A very small quantity of *Sym*-diphenylhydrazodithiodicarbonamide also separated.

The reaction with disubstituted thiosemicarbazides was also tried, but with little success. When sulphur monochloride in benzene was added to the tautomeric form of 1:4-phenyl-*p*-tolylthiosemicarbazide (stable, m.p. 176°), obtained by dissolving the thiosemicarbazide in a calculated quantity of potash, much decomposition occurred with formation of tar. If the reaction was conducted in chloroform without alcoholic potash, copious fumes of hydrogen chloride were evolved and the only product was a snuff coloured amorphous substance (of a high indefinite m.p.) precipitated by xylene from the pyridine solution of the crude product.

From 1:4-diphenylthiosemicarbazide an amorphous product free from sulphur, m.p. above 200° and having nitrogen 17·6 per cent, was obtained by carrying out the reaction under similar conditions. Thus with 1 : 4-disubstituted thiosemicarbazides, unlike 4-monosubstituted, the reaction is complicated and the amorphous products may not even be ring compounds, but open chain compounds of very high molecular weight, as shown by their high melting points. The diminished basicity of 1 : 4-disubstituted thiosemicarbazides having a negative group like phenyl in the 1-position may be responsible for preventing ring closure (cf. Guha, *J. Amer. Chem. Soc.*, 1922, **44**, 1502).

Unsymmetrical thiodiazoles of structure,



III

however, are not known : it appeared likely that these might also be obtained by the action of mild oxidising agents on dissimilarly substitued hydrazodithiodicarbonamides of the general formula, NHR·CS·NH·NH·CS·NHR′. Only one such carbonamide has been prepared by From, Soffner and Frey (*Annalen*, 1923, **434**, 285). In the course of this investigation several similar derivatives have been prepared and converted into thiodiazoles of the general formula III, by the action of iodine ; these, like I and II, also form diacetyl derivatives. During study of this reaction it was observed that boiling pyridine converts all 1, 6-disubstituted hydrazodithiodicarbonamides into the corresponding thiodiazoles.

EXPERIMENTAL

Interaction of the Potassium Derivative of 4-Phenylthiosemicarbazide and Sulphur Monochloride. Formation of 2 : 5-Diphenyldiimino-2 : 3 : 4 : 5-tetrahydro-1 : 3 : 4-thiodiazole and Sym-Diphenylhydrazodithiodicarbonamide.

4-Phenylthiosemicarbazide (6 g.) was dissolved in 25 c.c. of absolute alcoholic potash containing 8·036 g. per 100 c.c. and while stirred a solution of sulphur monochloride (3 g.) in 25 c.c. of dry benzene was gradually added ; an orange colour changing to yellowish white developed. Stirring was continued for 15 minutes more and the liquid filtered through a fluted paper was pink. The residue was washed on the paper three times with hot absolute alcohol and the collected filtrates concentrated to one-third yielded on cooling a small amount of solid ; on crystallisation from boiling alcohol or pyridine diluted with water it melted at 184° with effervescence (Found : C, 55·58 ; H, 4·45 ; N, 19·27 ; S, 21·16. $C_{14}H_{14}N_4S_2$ requires C, 56·00 ;

H, 4·00; N, 18·66; S, 21·33 per cent.). The compound is easily soluble in dilute alkalis and readily loses sulphur when treated with iodine in a suitable solvent; it is identical with *Sym*-diphenylhydrazodithiodicarbonamide.

The mother liquor was further concentrated and cooled, yielding a solid which crystallised from alcohol or dilute acetone in lustrous plates, m.p. 247° (Found: C, 62·75; H, 4·21; N, 19·8; S, 12·2. $C_{14}H_{12}N_4S$ requires C, 62·70; H, 4·47; N, 20·8; S, 11·9 per cent.). The compound was identified with 2,5-diphenylimino-2:3:4:5-tetrahydro-1:3:4: thiodiazole (Guha, *J. Amer. Chem. Soc.*, 1923, **45**, 1036); From, Layer and Nerz (*loc. cit.*), however, give 243° while Busch and Schmidt (*Ber.*, 1913, **46**, 2240) give 240° as the m.p. of the thiodiazole. The substance is soluble in hot alcohol, acetone, pyridine, but sparingly in ether and chloroform. The *diacetyl* derivative from acetic anhydride was crystallised from alcohol and melted at 225° (Found: N, 16·68. $C_{18}H_{16}O_2N_4S$ requires N, 16·00 per cent.).

4-p-*Tolylthiosemicarbazide and Sulphur Monochloride.*

4-*p*-Tolylthiosemicarbazide from *p*-tolyl mustard oil and hydrazine hydrate melts at 139–140° after several crystallisations from alcohol; Guha and Ray (*J. Amer. Chem. Soc.*, 1925, **47**, 385) give 172°, while Busch and Ulmer (*Ber.*, 1902, **35**, 1744) obtaining it by an indirect method give 134–135°.

4-*p*-Tolylthiosemicarbazide (6·5 g.) was dissolved in 25 c.c. of the specified alcoholic potash and treated with sulphur monochloride (3 g.) in 25 c.c. of dry benzene; *sym*-di-*p*-tolylhydrazodithiodicarbonamide was one of the products and melts at 198° with gas evolution (Found: N, 16·4; S, 19·0. $C_{16}H_{18}N_4S_2$ requires N, 17·0; S, 19·9 per cent.).

2 : 5-Di-*p*-tolyldi-imino-2 : 3 : 4 : 5-tetrahydro-1 : 3 : 4-thiodiazole was separated from the above hydrazo-compound by the procedure adopted previously, and after crystallisation from alcohol melts at 248° (Found: N, 18·56; S, 10·98. $C_{16}H_{16}N_4S$ requires N, 18·91; S, 10·81 per cent.). The *diacetyl* derivative melts at 172° (Found: N, 15·25. $C_{20}H_{20}O_2N_4S$ requires N, 15·13 per cent.), but Guha (*loc. cit.*) gives 235°.

4-o-*Tolylthiosemicarbazide and Sulphur Monochloride.*

With 4-*o*-tolylthiosemicarbazide (m.p. 145°) the reaction gave two products which were separated as already described; *sym*-Di-*o*-tolylhydrazodithiodicarbonamide melts at 175°, From, Soffner and Frey (*Annalen*, 1923, **434**, 285) giving 179° (Found: N, 17·3; S, 19·72. $C_{16}H_{18}N_4S_2$ requires N, 17·0; S, 19·9 per cent.). The second product, 2 : 5-di-*o*-tolyldi-imino-2 : 3 : 4 : 5-tetrahydro-1 : 3 : 4-thiodiazole, crystallises from alcohol and melts at 222°, and appears to be new (Found: N, 18·86; S, 10·25. $C_{16}H_{16}N_4S$ requires N, 18·91; S, 10·81 per cent.).

The *diacetyl* derivative crystallises from alcohol and melts at 257° (Found: N, 1505. $C_{20}H_{20}O_2N_4S$ requires N, 15·13 per cent.).

4-*Phenylthiosemicarbazide and Sulphur Monochloride at varying Temperatures.*

(*a*) *In a freezing mixture.*—A solution of 4-phenylthiosemicarbazide (6 g.) in 25 c.c. of the specified alcoholic potash was cooled in the freezing mixture while stirred, and a solution of sulphur monochloride (3 g.) in anhydrous benzene admitted drop by drop. When the reaction seemed completed, the pink solution was filtered from sulphur and potassium chloride. After washing the solid several times with alcohol the collected filtrates were concentrated and cooled in a freezing mixture, when a white solid separated and crystallised from alcohol in plates melting at 247°; a second crop had the same m.p.

(*b*) At 27°.—An almost quantitative yield of *sym*-diphenylhydrazodithiodicarbonamide, m.p. 184°, was obtained without a trace of the thiodiazole.

Action of boiling Pyridine on sym-*Diphenylhydrazodithiodicarbonamide.*

The hydrazo-compound dissolved in boiling pyridine gave a clear solution which on cooling deposited much less solid than was originally taken, and was found to be unchanged substance. On cooling the mother liquor with ice nothing separated, but dilution with water gave a precipitate which, when recrystallised from alcohol, melted at 246° and was found to be 2 : 5-diphenyldiimino-2 : 3 : 4 : 5-tetrahydro-1 : 3 : 4-thiodiazole.

4-*Phenylthiosemicarbazide and Sulphur Monochloride in Chloroform.*

4-Phenylthiosemicarbazide (16·7 g.) heated under reflux with 600 c.c. of dried chloroform gave an almost clear solution; to this was added a solution of sulphur monochloride (6·75 g.) in 25 c.c. of dry chloroform, with shaking, and allowed to remain for some time with a calcium chloride guard-tube. The faint yellow solution was then filtered from about 9–10 grams of 4-phenylthiosemicarbazide hydrochloride, and after most of the solvent was distilled, there remained a thick oil smelling of phenyl mustard oil; treatment with ether gave *sym*-diphenylhydrazodithiodicarbonamide melting at 184°.

2 : 5-*Phenyl*-o-*tolyldiimino*-2 : 3 : 4 : 5-*tetrahydro*-1 : 3 : 4-*thiodiazole.*

4-Phenylthiosemicarbazide and *o*-tolyl mustard oil (molecular proportion) in boiling alcohol gave 1 : 6-phenyl-*o*-tolylhydrazodithiodicarbonamide which, crystallised from dilute pyridine, melts at 179°. The oxidation was conducted according to the method of From, Layer and Nerz (*loc. cit.*), an alcoholic solution of iodine being added in small portions to 1 : 6-phenyl-*o*-tolylhydrazodithiodicarbonamide (7 g.) in 80 c.c. of 95 per cent. boiling alcohol under reflux. Addition of iodine was stopped when a permanently brown and almost clear solution was obtained. The filtrate from sulphur gave a copious white precipitate of the thiodiazole with caustic potash and this when crystallised from 95 per cent. alcohol melts at 200° (Found : N, 20·5. $C_{15}H_{14}N_4S$ requires N, 19·9 per cent.). The *diacetyl* derivative was crystallised from alcohol, m. p. 223° (Found: N, 15·92. $C_{19}H_{18}O_2N_4S$ requires N, 15·3 per cent.).

2 : 5-*Phenyl*-m-*tolyldiimino*-2 : 3 : 4 : 5-*tetra-hydro*-1 : 3 : 4-*thiodiazole.*

1 : 6-Phenyl-*m*-tolylhydrazodithiodicarbonamide prepared from 4-phenylthiosemicarbazide and *m*-tolyl mustard oil melts at 180°. The thiodiazole obtained from this by oxidation as described in the last case melts at 216°, when crystallised from alcohol (Found: N, 20·3. $C_{15}H_{14}N_4S$ requires N, 19·9 per cent.). The *diacetyl* derivative crystallised from alcohol melts at 177° (Found : N, 15·69. $C_{19}H_{18}O_2N_4S$ requires N, 15·3 per cent).

2 : 5-o-*Tolyl*-m-*tolyldiimino*-2 : 3 : 4 : 5-*tetrahydro* 1 : 3 : 4-*thiodiazole.*

1 : 6-*o*-Tolyl-*m*-tolylhydrazodithiodicarbonamide prepared from 4-*o*-tolylthiosemicarbazide and *m*-tolyl mustard oil melts at 166°. The thiodiazole obtained by oxidation melts at 190° when crystallised from alcohol ; the *diacetyl* derivative melts at 231° (Found : N, 14·75. $C_{20}H_{20}O_2N_4S$ requires N, 14·73 per cent.).

2 : 5-m-*Xylyl*-m-*tolyldiimino*-2 : 3 : 4 : 5-*tetrahydro*-1 : 3 : 4-*thiodiazole.*

1 : 6-*m*-Xylyl-*m*-tolylhydrazodithiodicarbonamide prepared from 4-*m*-xylylthiosemicarbazide (m.p. 142°) and *m*-tolyl mustard oil melts at 161°. The thiodiazole obtained by oxidation melts at 190° ; the *diacetyl* derivative melts at 263° (Found : N, 13·93. $C_{21}H_{22}O_2N_4S$ requires N, 14·21 per cent.).

2 : 5-o-*Tolyl*-m-*xylyldiimino* 2 : 3 : 4 : 5-*tetrahydro*-1 : 3 : 4-*thiodiazole.*

1 : 6-*o*-Tolyl-*m*-xylylhydrazodithiodicarbonamide obtained from 4-*o*-tolylthiosemicarbazide and *m*-xylyl mustard oil (b.p. 258°), melts at

164°. The thiodiazole crystallises from alcohol and melts at 205°; the *diacetyl derivative* melts at 224° (Found: N, 14.33. $C_{21}H_{22}O_2N_4S$ requires N, 14·21 per cent.).

*2 : 5-Phenyl-*m*-xylyldiimino-2 : 3 : 4 : 5-tetrahydro-1 : 3 : 4-thiodiazole.*

1:6-Phenyl-*m*-xylylhydrazodithiodicarbonamide from 4-phenylthiosemicarbazide and *m*-xylyl mustard oil melts at 171°. The thiodiazole crystallised from alcohol melts at 189°; the *diacetyl derivative* crystallised from alcohol melts at 120° (Found: N, 14·73. $C_{20}H_{20}O_2N_4S$ requires N, 14·73 per cent.).

SUMMARY.

Interaction of sulphur monochloride and 4-substituted thiosemicarbazides gives thiodiazoles along with small quantities of hydrazodithiodicarbonamides.

Several new mixed hydrazodithiodicarbonamides have been prepared and oxidised to the corresponding thiodiazoles.

Pyridine has been found to act as a ring closing agent on hydrazodithiodicarbonamide and its derivatives.

Department of Organic Chemistry,
Indian Institute of Science,
Bangalore.

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I.—POWER ALCOHOL AND PAPER FROM RICE STRAW.

By D. D. Deshpande.

Although numerous cellulosic materials have been examined as possible sources of power alcohol, very little systematic work has been conducted on rice straw, millions of tons of which annually go to waste in India alone. The straw, having low nutritive value, is not fed to cattle in many parts of India ; its use in making manure involves much loss of dry matter and can be hardly regarded as economical.

Experiments on the commercial production of alcohol from rice straw were carried out in Burma, and a process involving hydrolysis of straw pulp with either hydrochloric acid vapour or atomised calcium hypocholorite and chlorine has been patented (Rogers and Bedford, 1919, *E. P.* 144079). Takatomi (*J. Soc. Chem. Ind. Japan*, 1926, **29**, 43) observed that, when heated with one per cent. sulphuric acid at about 134° for 30 minutes in the autoclave, rice straw yields 15 per cent. of sugar on the dry weight, giving on fermentation 5·4 per cent. of alcohol. Sen (*Institution of Chemists, India*, 1926, Publcn. 1) obtained 4·3 per cent. by weight of alcohol from rice straw after hydrolysis with dilute sulphuric acid.

The object of the present investigation, undertaken independently of those of Takatomi and Sen, was to standardise the conditions of hydrolysing rice straw with sulphuric acid so as to obtain (*a*) from the hydrolysate, by fermentation with yeast alone, or supplemented by other organisms, a high yield of liquid fuel and (*b*) from the residue, a large yield of cellulosic matter which could be converted into paper pulp of good quality.

EXPERIMENTAL.

The rice straw was obtained locally and had the following percentage composition :—

Moisture, 7·05 ; fat, 1·05 ; protein, 1·70, ash, 15·95 ; sucrose, 0·18 ; starch and hemicellulose, 16·24 ; furfuroids as pentoses, 25·53 ; and crude fibre (celluloses, lignin, etc.), 36·00.

Digestion.—The straw (100 g.) was cut into lengths of 3 to 4 inches and in lead pots digested with dilute sulphuric acid under a variety of conditions. The time required to raise the pressure in the autoclave averaged 1½ minutes for every 15 lbs. When digestion was complete, the pressure was released at the rate of one atmosphere in 5 minutes. The hydrolysed straw was drained, extracted with hot water three or four times and finally pressed in a wooden screw-press; the extract was neutralised with pure precipitated chalk, filtered through kieselguhr and concentrated.

Reducing material was estimated by the Bertrand method, and the remaining liquid at suitable concentration was fermented with acclimatised yeast, yeast-water being added in small quantity to serve as additional nutrient. The fermentation generally lasted more than three days when the reducing material in the residual mash was estimated and the quantity of fermented sugar determined by difference from the amount originally present. The yield of alcohol and the quantity of pentose in the medium were determined only in some cases; the former by density and the latter by the method of Powell and Whittekar (*J. S. C. I.*, 1924, **43**, 35 T). A strain of yeast from the local breweries was used, and after much initial difficulty was acclimatised to the straw extract. The fermentations took place at 25–27°.

Acid concentration and ratio to straw.

Some digestions were conducted with different concentrations of sulphuric acid, but keeping the total quantity of sulphuric acid (10 g.), pressure (4 atmos.) and time constant (Table I). In this and the following tables the reducing matter is expressed as grams of glucose from 100 g. of straw.

TABLE I.

Volume of acid, c. c.	Time of digestion, mins.	Total reducing material	Fermentable sugars
500	30	22·90	6·39
750	,,	23·22	6·45
1,000	,,	23·39	6·80
500	60	22·35	5·93
750	,,	22·70	5·98
1,000	,,	23·05	6·04

The results show that the extent of hydrolysis does not depend so much on the concentration of acid as on the ratio of acid to straw. The observations are in accordance with those of Kressman (*Dept. Agric. U. S. A. Bull.*, 1922, **983**, 1) on the hydrolysis of wood-waste. As increased volume of acid did not appreciably improve the yield of either the total reducing material or the fermentable sugars, the volume used in subsequent experiments was fixed at 500 c.c. only; the small gain from using a much larger volume of acid would not compensate for the loss of energy and time required to concentrate the increased quantity of extract. It is possible that the apparently lower yield of reducing matter on using more concentrated acid was due to some caramelisation of sugar.

Effect of steeping the straw in acid for varying periods.

Straw (100 g.) was steeped in 500 c.c. of 1 per cent. acid for varying periods; the reducing sugars were then extracted and estimated (Table II).

TABLE II.

Time of steeping, hrs. ...	41	68	116	232	277
Total reducing material ...	0·64	0·64	0·74	1·15	1·16

Thus the cold acid appears to have no action on straw.

Digestion after prolonged steeping.—Straw was steeped in cold acid and then digested at 4 atmospheres for half-an-hour.

TABLE III.

Time of steeping, hrs. ..	0	12	24	48
Reducing material in the hydrolysate ...	23·15	20·90	20·13	20·00

Thus the yield of reducing sugars was highest when the straw was digested without previous steeping.

A series of digestions followed by fermentation trials were carried out with a view to determining the optimum conditions for a successful fermentation. The results are given in Table IV, concentrations of acid being expressed as percentages on the dry weight of straw.

TABLE IV.

Acid, 1·25 per cent.			Acid, 2·5 per cent.		
Time of digestion	Total reducing material	Fermentable sugars	Time of digestion	Total reducing material	Fermentable sugars
Pressure, 2 atmos.			Pressure, 2 atmos.		
½	0·30	0·00	½	2·25	0·76
1	0·82	0·24	1	3·27	0·91
2	1·62	0·73	2	4·51	0·45
3	2·06	0·68	3	4·96	0·41
5	2·77	0·81			

TABLE IV.—(*continued*).

Acid, 1·25 per cent.			Acid, 2·5 per cent.		
Time of digestion	Total reducing material	Fermentable sugars	Time of digestion	Total reducing material	Fermentable sugars
Pressure, 3 atmos.			Pressure, 3 atmos.		
½	1·36	0·43	½	3·45	1·15
1	1·83	0·67	1	3·80	1·30
2	2·45	0·70	2	6·87	2·45
3	2·50	0·76	3	8·18	1·09
Pressure, 4 atmos.			Pressure, 4 atmos.		
½	1·68	0·73	½	5·38	2·04
1	2·42	1·14	1	4·81	0·50
2	3·19	1·33			
3	3·95	1·39			
Pressure, 5 atmos.			Pressure, 5 atmos.		
½	2·09	0·65	½	7·26	1·17
1	2·87	0·98	1	7·44	0·93
2	3·99	1·64			
3	5·46	1·61			
Pressure, 6 atmos.			Pressure, 6 atmos.		
½	3·28	1·03	½	10·36	...
1	5·90	1·26	1	10·27	...
			Pressure, 7 atmos.		
2	6·84	1·39	¼	8·94	...
3	5·17	0·36	½	10·10	...

Acid, 5·0 per cent.			Acid, 10·0 per cent.		
Time of digestion	Total reducing material	Fermentable sugars	Time of digestion	Total reducing material	Fermentable sugars
			Pressure, 1 atmos.		
			½	9·50	1·52
Pressure, 2 atmos.			Pressure, 2 atmos.		
1	13·24	2·11	½	18·82	4·65
Pressure, 3 atmos.			Pressure, 3 atmos.		
1	18·11	3·29	¼	18·22	4·18
			½	20·93	4·77
Pressure, 4 atmos.			Pressure, 4 atmos.		
½	16·90	1·93	¼	21·27	5·06
1	19·32	3·71	½	22·59	5·83
2	19·95	4·14	1	21·25	5·52
Pressure, 5 atmos.			Pressure, 5 atmos.		
¼	18·72	3·14	¼	22·70	5.94
½	19·66	5·56	½	20·73	5·76
1	20·59	6·22			
2	19·26	3·66			
Pressure, 6 atmos.					
¼	19·48	2·97			
½	20·21	4·96			
1	19·58	3·94			

The results show that with low concentration of acid the total reducing material increases steadily with time. Thus, at a pressure of two atmospheres the total reducing material formed at the end of 5 hours was nearly 9 times that formed after digestion for only 30 minutes. At higher pressures, on the other hand, the maximum yield of sugar was attained in a very short period, more prolonged digestions resulting in reduced yields. There was marked increase in both total reducing material and fermentable sugar when acid of 5 per cent. and above was used, the highest yields being obtained by digesting at 4 to 5 atmos. for 15–30 minutes,

Identification of sugars in the hydrolysate.

Rice straw (100 g.) was digested with 500 c.c. of 2 per cent. sulphuric acid at 4 atmos. for 30 minutes. The extract was neutralised with barium carbonate, filtered, concentrated at 25 mm. and treated with 3 times its volume of 95 per cent. alcohol, the precipitate being allowed to settle over night.

The filtrate was concentrated in a vacuum and the residual syrup dissolved in methyl alcohol, boiled with norit, filtered through kieselguhr and concentrated in a vacuum. The sticky product was dark and could not be crystallised.

Oxidation of the syrup by bromine was conducted by the method of Fischer (*Ber.*, 1889, **22**, 3218). Analysis of the mixed calcium salts showed that the syrup consisted only of pentoses and hexoses, the former preponderating. The syrup was examined qualitatively for sugars, the phenylhydrazine reaction for arabinose and mannose, and the mucic acid test for galactose being negative. Xylose was identified by the double salt of cadmium xylonate and cadmium bromide (Bertrand, *Bull. Soc. Chim.*, 1891, **5**, 554 ; 1896, **15**, 592). By oxidizing the syrup with nitric acid and neutralising the product with potassium carbonate according to Fischer and Piloty (*Ber.*, 1891, **24**, 527), potassium saccharate was obtained, indicating glucose. Since no other carbohydrate could be detected, it was inferred that the only sugars in the hydrolysate were glucose and xylose.

Yield of alcohol from the hydrolysates and determination of xylose.— To determine whether the yield of alcohol corresponded to the amounts of sugar fermented, some trials were made under conditions already found to be the most satisfactory. The free furfural in the hydrolysate after digestion, and the xylose in the liquor after fermentation were determined by the method of Powell and Whittekar (*loc. cit.*) The results have been presented as percentages on the dry weight of straw (Table V).

TABLE V.

Pressure, atmos.	Acid per cent.	Time of digestion, hrs.	Reducing material as glucose	Fermentable sugars as glucose	Free furfural	Alcohol	Xylose
5	5	½	19·50	5·38	1·15	2·50	13·82
5	5	1	20·50	6·20	1·30	3·00	13·62
4	10	½	22·99	6·05	1·21	2·85	15·65
4	10	1	20·90	5·26	1·37	2·51	13·62
5	10	1¼	22·60	5·90	1·24	2·62	15·68
5	10	½	21·45	5·71	1·38	2·65	15·01

The yields of alcohol were consistently lower than those expected for complete fermentation of the sugars to alcohol and carbon dioxide; the free furfural in the hydrolysate probably had a deleterious effect on the yeasts. The nonfermentable sugar in the residue consisted almost exclusively of xylose.

Effect of previously boiling the straw with water.—The straw was boiled for about 12 hours, the water being changed once in two hours, until the extract was clear and colourless. The residue after pressing and drying was digested with acid and the extract fermented as in the previous experiment. The results are presented in Table VI.

TABLE VI.

Pressure, atmos.	Acid per cent.	Time of digestion, hrs.	Reducing material as glucose	Fermentable sugars as glucose	Free furfural	Alcohol	Xylose
5	5	½	24·5	3·65	1·98	1·55	19·58
5	5	1	24·67	7·46	2·15	3·50	16·23
4	10	½	24·67	5·44	1·58	2·51	19·42
4	10	1	23·80	6·28	1·81	3·01	17·53
5	10	¼	25·97	11·26	1·96	5·22	14·53
5	10	½	25·55	7·26	1·79	3·45	18·43

Thus the yields generally exceeded those with unboiled straw, the highest corresponding to 11·25 gallons of 95 per cent. alcohol per ton of straw.

Effect of concentrating the hydrolysate in vacuo.—The straw (200 g.) was digested with 10 per cent. sulphuric acid at 4 atmos. for half-an-hour, the extract being pressed, neutralised and filtered. One half of the filtrate was concentrated on boiling water, the other at 70° *in vacuo*, and analysed for reducing fermentable sugars, furfural and alcohol on fermentation (Table VII). The distillate obtained during concentration gave furfural corresponding to 5·01 per cent. on the weight of the straw.

TABLE VII.

Temp. of concentration	Total reducing sugar matter	Fermentable sugar	Furfural	Alcohol
Boiling	23·79	6·59	traces	3·1
70°	22·43	7·57	nil	3·65

Thus by concentrating *in vacuo* the furfural was separated and the fermented extract gave a slightly higher yield of alcohol.

Fermentation of straw extract by B. Acetoethylicus.

From the foregoing experiments it was inferred that (*a*) fermentation by yeast alone could not lead to satisfactory yields of alcohol and (*b*) most of the reducing matter unfermented by yeast was xylose. Attempts were therefore made to ferment the straw extract by organisms known to be capable of fermenting pentoses. Thayson and Galloway observed (*Ann. Appl. Biol.*, 1928, **15**, 392) that *B. Acetoethylicus* could ferment the hydrolysates of rice husk and straw yielding 14 and 19 gallons respectively of alcohol-acetone mixture; some trials were therefore made to ascertain whether the same organism could ferment the residue after the yeast fermentation.

The straw was hydrolysed by steaming in the manner described by Thayson and Galloway (*loc. cit.*), and the extract contained 9·10 per cent. of reducing matter not fermentable by yeast. The culture of *B. Acetoethylicus*, originally obtained in the form of spores in sand, was raised on the maize mash medium (Northrop, Ashe and Senior, *J. Biol. Chem.*, 1919, **39**, 1), and showed all the cultural characteristics described by Thayson and Galloway (*loc. cit.*). By adding small quantities of cooked maize and peptone with excess of calcium carbonate to the straw hydrolysate, repeated sub-culturing acclimatized the organism to the medium. The fermentation generally began after 24 hours and occupied about a week; incubations were at 37°.

Substitutes for peptone.—This being rather costly, a substitute was sought in other nitrogenous compounds. Straw extracts containing 1 per cent. each of (*a*) ammonium phosphate, (*b*) dihydrogen sodium phosphate, (*c*) peptone and (*d*) ammonium phosphate were inoculated with active cultures of the organism and the progress of fermentation studied. Extract containing 20 per cent. by volume of yeast water was also tried as a nutrient.

It was observed that the fermentation in presence of ammonium phosphate and yeast water began even more vigorously than with peptone and concluded in eight days instead of nine. In extracts containing other compounds the fermentation began more slowly and required about ten days for completion.

Fermentation of straw extract.

Ammonium phosphate (1 per cent.) and yeast water (20 per cent. by volume) were added to straw extract (*a*) when fresh, and (*b*) after yeast fermentation, the sterilised extracts inoculated with *B. Acetoethylicus* and incubated at 37°. The products obtained after complete fermentation were determined (Table VIII).

TABLE VIII.

Extract	Period of fermentation, days	Total Sugar	Sugar fermented	Fuel mixture as alcohol
Unfermented + ammonium phosphate ...	8	5·32	4·58	1·91
Unfermented + yeast water ...	10	5·32	4·60	1·82
Fermented + ammonium phosphate ...	8	5·08	4 76	2·01
Fermented + yeast water.	10	5·08	4·42	1·72

The results suggest that *B. Acetoethylicus* can ferment the residual pentoses and the fresh straw extract equally well. Further work to determine whether the organism can be used economically to supplement fermentation by yeast is in progress.

Fermentation by cellulose-decomposing organisms.

Vilgeon, Fred and Peterson (*J. Agric. Sci.*, 1926, **16**, 1) having shown that certain organisms present in actively decomposing manure are capable of fermenting 70–95 per cent. of cellulose, attempts were made to ferment the straw with thermophylic bacteria from raw sewage, horse-dung and farmyard manure. Straw which was (*a*) untreated, (*b*) boiled with water and (*c*) treated with 1 and 5 per cent. alkali respectively, was inoculated with mixed flora from the different sources and incubated at 65°.

Alkali-treated straw was fermented actively by the organisms from dung and farmyard manure; when action was complete after about ten days the products were distilled, yielding mainly butyric acid in all the cases. Only traces of alcohol were obtained.

Mechanism of acid hydrolysis.

With a view to (*a*) determining the extent to which the different constituents of straw were affected by acid hydrolysis, and (*b*) standardising the conditions to obtain the highest yield of cellulosic residue for making paper pulp, the distribution of constituents was studied by the method of Raitt (*Indian For. Records*, 1913, **5**, pt. 3).

3

Moisture was determined by drying at 110° for 8 hours. Water-soluble constituents were removed by boiling for 12 hours, the water being changed every two hours till the extract was colourless, the residue being pressed in a hydraulic press and dried. Resins, fats, waxes, pectins and allied materials, together with water-soluble constituents were removed by boiling with 1 per cent. sodium hydroxide for 6 hours. The residue was washed with hot followed by cold water till the washings gave no colour with phenolphthalein, then pressed and dried. Almost all the constituents except cellulose were removed by digesting the straw with 5 per cent. sodium hydroxide at 130° for 6 hours. The residue was washed with water, pressed and dried.

The solid matter left after the different treatments was as follows:—drying at 110°, 92·95 (*a*): boiling with water, 72·65 (*b*); boiling with 1 per cent. alkali, 46·4 (*c*); and digesting with 5 per cent. alkali at 130°, 38·7 (*d*). The percentages of moisture, ash and total nitrogen in the above were determined (Table IX).

TABLE IX.

	Untreated	*b*	*c*	*d*
Moisture	7·05	7·4	8·00	8·00
Ash	15·95	11·07	7·29	5·68
Total nitrogen ...	0·27	0·21	0·06	0·04

Thus the amounts of ash and nitrogen diminish on passing from one treatment to another.

The insoluble matter from the different treatments was digested with sulphuric acid as detailed in Tables V and VI, and fermented, conditions of fermentation and methods of examining the products being also the same. The results were so calculated as to present the amounts in grams of reducing sugars, furfural, alcohol and xylose contributed by (A) untreated straw, (B), (C) and (D) water-soluble, 1 per cent. alkali-soluble and 5 per cent. alkali-soluble materials, respectively, and (E), the residue after 5 per cent. alkali treatment, the starting material being, in each case, 100 grams of untreated straw. Since 1 per cent. alkali extracted materials also dissolving in water, those dissolving in the alkali alone following an extraction with water were calculated by subtracting the former from the latter. In like manner the products derived from the 5 per cent. alkali extract following an extraction with 1 per cent. alkali were calculated by subtracting the total 1 per cent. alkali-soluble materials from the total 5 per cent.

alkali-soluble ones. In the following tables, B includes starch and its transformation products, sugars, soluble gums, tannins, colouring matter and other water-soluble materials; C, resins, fats, waxes, gums insoluble in water and pectins; D, mostly lignin, mixed with some β-cellulose; and E, α-cellulose and some β-cellulose (Raitt, *loc. cit.*). The substances derived from them and their products of hydrolysis are shown in Table X.

TABLE X.

Acid, 5 per cent., Pressure, 5 atmos., Time of digestion, 30 mins.

Material	Reducing sugars as glucose	Furfural	Alcohol	Fermentable sugars as glucose	Xylose
A	19·50	1·15	2·50	5·38	13·82
B	1·70	nil	1·37	2·73	nil
C	7·64	0·93	nil	0·20	7·07
D	6·70	0·37	0·92	1·90	4·18
E	3·46	0·14	0·24	0·55	2·97

Acid, 5 per cent., Pressure, 5 atmos., Time of digestion, 1 hour.

A	20·50	1·30	3·00	6·20	13·62
B	2·58	nil	0·46	0·78	1·83
C	7·55	0·85	0·92	2·06	...
D	5·98	0·09	1·10	2·22	3·30
E	4·39	0·62	0·52	1·14	3·34

Acid, 10 per cent., Pressure, 4 atmos., Time of digestion, 30 mins.

A	22·99	1·21	2·85	6·05	15·65
B	5·07	0·06	1·03	2·10	1·55
C	7·19	0 52	0·41	1·08	6·16
D	4·82	0·45	0·63	1·21	3·71
E	4·91	0·18	0·78	1·66	4·23

TABLE X—(*continued.*)

Material	Reducing sugars as glucose	Furfural	Alcohol	Fermentable sugars as glucose	Xylose
Acid, 10 per cent., Pressure, 4 atmos., Time of digestion, 1 hour.					
A	20·90	1·37	2·51	5·26	13·62
B	3·61	0·05	0·32	0·70	0·88
C	6·89	0·58	0·57	1·11	6·48
D	3·90	0·36	0·47	1·09	2·10
E	6·50	0·38	1·15	2·36	4·16
Acid, 10 per cent., Pressure, 5 atmos., Time of digestion, 15 mins.					
A	22·60	1·24	2·62	5·90	15·68
B	3·73	nil	1·17	2·27	...
C	8·79	0·83	2·69	4·73	...
D	4·50	0·40	0·40	0·88	5·49
E	5·58	0·19	0·70	1·56	1·95
Acid, 10 per cent., Pressure, 5 atmos., Time of digestion, 30 mins.					
A	21·45	1·38	2·65	5·71	15·01
B	2·88	0·08	0·14	0·44	1·62
C	7·75	0·49	0·93	1·86	6·09
D	4·13	0·52	0·52	1·08	3·02
E	6·69	0·29	1·06	2·33	4·28

The figures show that the total reducing sugars increased with concentration of acid, pressure and time of digestion, the enhanced yields appearing to have been derived mainly from the water-soluble materials (B) and the residue after 5 per cent. alkali digestion. The 1 per cent. alkali-soluble materials however, yielded the largest amounts, the 5 per cent. alkali-soluble generally coming next. Reducing sugar from the former consisting exclusively of xylose had very little value for yeast fermentation. Those from the water-soluble fractions, particularly when obtained at the lower pressures and after shorter periods of digestion, were mainly fermentable and contained very small amounts of xylose at the greater concentrations of acid, higher pressures and more prolonged digestions, the water-soluble fraction contained increasing amounts of the pentose.

The major part of the furfural was derived from the 1 per cent. alkali extract, but the quantities recorded cannot be taken to represent the total amounts formed during the digestions: quite appreciable amounts may have been lost during release of pressure and cooling of autoclave. While indicating the presence of large amounts of unfermentable pentosans, free furfural of the concentrations indicated does not appear to have had any direct effect on the yields of alcohol.

The quantities of fermentable sugar in the hydrolysates and the corresponding yields of alcohol were closely related to each other except in one instance, when removal of the water-soluble materials led to an increased yield of alcohol. Taking them together, it may be noted that, whereas the water-soluble materials contained appreciable amounts of fermentable sugars and yielded corresponding quantities of alcohol when the digestion was carried out at low pressure and for short periods, they yielded less of those products when the digestion was carried out at higher pressure or for long periods. The reducing sugars in the hydrolysates increased in the latter, but they were mostly unfermentable. With increased concentration of acid, greater pressure and prolonged digestion, the more resistant forms of carbohydrates such as were extracted by 5 per cent. alkali and the cellulosic residue underwent hydrolysis resulting in small quantities of fermentable sugars together with varying amounts of xylose. When the yield of fermentable sugars from the resistant forms increased, that from the water-soluble forms decreased : it is possible that the latter, being the first in solution, were acted upon by the strong acid during the long periods of digestion at high pressure and hydrolysed to non-fermentable forms.

Furfuroids were extracted by all the treatments in varying quantities, these being generally the largest in the 1 per cent alkali extract, suggesting that they were present mainly in the form of pentosans in rice straw. The 5 per cent. alkali extract and the cellulosic residue yielded appreciable amounts of furfuroids only on raising the concentration of acid and prolonging the time of digestion, thereby suggesting that the latter were originally present in forms highly resistant to acid hydrolysis.

The formation of fairly large amounts of furfural on hydrolysing the residue from digestion with 5 per cent. alkali suggests that this was cellulose mixed with varying amounts of other materials. Celluloses are essentially hexosans, and since only negligible amounts of fermentable hexoses were formed from the residues under the different conditions of digestion, it may be inferred that cellulose of straw which will be required as the raw material for making paper pulp will be almost unaffected during hydrolysis.

To determine approximately the extent to which the residue after 5 per cent. alkali treatment contained furfuroids as impurities, 100 gram lots of (*a*) the residue, (*b*) a good specimen of bleached bisulphite pulp and (*c*) filter paper were digested with 10 per cent. sulphuric acid at 5 atmos. for 15 minutes, the total reducing materials, fermentable sugars and xylose in the hydrolysates being determined (Table XI).

TABLE XI.

Material	Reducing matter as glucose	Fermentable sugars as glucose	Xylose
Residue after 5 per cent. alkali treatment.	14·42	4·04	10·45
Bleached bisulphite pulp	6·80	4·90	1·93
Filter paper	5·42	4·96	0·44

Filter paper yielded only minute amounts of furfural on distillation with 12 per cent. hydrochloric acid, the slightly greater yield from the sulphite pulp indicating a small proportion of pentosans in the latter. The large quantities obtained from the straw residue show that the latter contained about 10 per cent. by weight of impurities in the form of furfuroids. The amounts of fermentable sugars obtained from the different materials were the same, indicating that the former were derived mainly from cellulose. The quantities of xylose in the hydrolysates, on the other hand, increased as the specimens became less pure.

Paper-pulp and Paper from Rice Straw.

Various forms of straw have long been used as sources of paper, the pulp when mixed with about 30 per cent. of another having long fibre, such as that of cotton or linen, being utilised for manufacturing different kinds of paper. Although vast amounts of straw go to waste, India imports enormous quantities of straw pulp, largely due to lack of precise details as to the previous treatment of straw and subsequent bleaching.

Trials at the Imperial Institute (*Bull. Imp. Inst.*, 1918, **16**, 21) showed that rice straw as a whole is a suitable material for certain kinds of paper and straw-board. The present investigation has shown that cellulose is mostly unaffected by acid hydrolysis of rice straw for fermentation, and the bulky residues pressed from the extracts appeared suitable for making paper-pulp. A study of some of the processes was therefore undertaken.

Pulp from residue after acid digestion.—This was treated with a bleaching liquor of 12 per cent. alkaline sodium hypochlorite; bleaching was rapid and on washing till neutral to phenolphthalein, the pulp, almost white, was 42 per cent. of the straw-weight. As already observed (*D. S. I. R.*, 1927, Mem. 4), addition of acid to the bleach was not required as the residue generally contained small quantities of acid.

Pulp from rice straw by alkali treatment.—The straw was treated with caustic soda of 3–6 per cent. under different pressures for 4½ hours. The results are given in Table XII.

TABLE XII.

Expt.	Caustic Soda per cent.		Pressure, lbs.	Dry pulp, per cent.
	on straw	in solution		
A	18	6	40	35
B	15	4	40	37
C	15	4	30	42
D	8	4	40	40
E	6	3	40	42

The product from all experiments was easily bleached to a creamy white, pulps from C and E requiring more bleach and longer treatment to equal the others in whiteness.

The foregoing pulps contained small particles of husk resistant to acid and alkali, and consequently quite fibrous; in latter trials these were removed before digestion, and a useful uniform pulp was thus obtained.

Pulp from rice straw by acid treatment.—Cleaned straw was digested under 4 atmos. with 10 per cent. sulphuric acid for 30 minutes, 60 per cent. by weight remaining after extraction; washing and bleaching gave 42 per cent. of good quality pulp.

The Deccan Paper Mills, Poona, reported favourably on the pulps obtained by acid and alkali treatment, mentioning that they were well suited to paper-making and have properties resembling those of imported pulps.

Preparation of paper from the pulp.—The samples obtained by acid and alkali treatment were sent to a hand-paper factory at Junnar, Bombay Presidency. As the pulp had been squeezed by hand in cloth, some knots which could not be well crushed or beaten by hand were formed after drying; in later trials knots were precluded by mechanical pressing and converting the pulp into sheets.

Straw pulp fibre being short, paper could be prepared from it only after admixture with 10–15 per cent. of long fibred pulp, waste paper being found useful for the purpose.

The same samples of pulp were used for making paper by hand. The dried 'half-stuff' (as the pulp is called before beating) containing knots was beaten in a beating machine for about 6 hours to separate the fibres into single units. It was then transferred to a big enamelled cylindrical iron vessel, and size (1 per cent. by weight of rosin soap and alum) added. By using a sample mould, 8″ × 10″ paper which did not shrink appreciably was obtained; the wet paper was transferred to a couch sheet by pressing the mould on it gently but uniformly. To remove water sufficiently to collect the wet paper by hand, further pressure between two couch sheets was applied in a screw press for two or three minutes when the paper was easily stripped, and hung to dry in 8–10 hours.

Some samples of paper were also prepared with filling materials such as starch, china-clay and calcium sulphate along with the sizing material.

The paper obtained from acid treated pulp was more bulky than the one obtained by digesting the straw with alkali; it had also the hardness and 'rattle' of writing paper. Omission of size gave a filter paper useful even for precipitates like barium sulphate. Ash from the paper amounted to 0·6–0·8 per cent. (acid treatment) and 5·6 per cent. (alkali treatment).

SUMMARY.

With dilute sulphuric acid as the hydrolysing agent, the best results were obtained by digesting the straw with 5 per cent. of acid calculated on the weight of straw and carrying out the digestion at 5 atmospheres for one hour, an extract containing 20·5 per cent. of total reducing sugars being obtained. Only about one third of the sugar was fermentable by yeast, the yield of alcohol being about 3 per cent. by weight if the straw was digested as such, rising to over 5 per cent. if boiled previously with water.

By concentrating the hydrolysate *in vacuo*, furfural amounting to about 5 per cent. by weight was obtained in the distillate ; a slightly enhanced yield of alcohol was also noticed.

The sugar left from alcoholic fermentation was mainly xylose, fermentable by *B. Acetoethylicus* when a further yield of alcohol with acetone was obtained, amounting to about 2 per cent. by weight of liquid fuel.

Digestion trials with the different constituents of the straw showed that (*a*) xylose was derived mainly from the alkali-soluble constituents and (*b*) cellulose was the least affected by acid digestion.

The undecomposed portion amounted to about 60 per cent. of the total straw-weight ; being largely cellulose, it was converted into paper-pulp of good quality.

By carrying out the acid hydrolysis under favourable conditions, fermenting the hydrolysate with yeast and *B. Acetoethylicus* in succession and utilising the undigested residue for making paper-pulp, 20–25 gallons of liquid fuel which is mostly alcohol, and 8 cwts. of good quality pulp may be obtained from every ton of rice straw.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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II.—ESTIMATION OF PENTOSES AND PENTOSANS BY DIFFERENT METHODS.

By D. D. Deshpande.

In the foregoing investigation, the author made a comparative study of different methods commonly used for estimating pentoses and pentosans in straw. There is no method at present for direct estimation; those in use depend on the quantitative transformation of the carbohydrates, by distilling with acid, into furfural which can be estimated in various ways. Since small amounts of furfural are formed also from other carbohydrates, none of the methods can be regarded as specific for pentoses and pentosans.

Distillation of furfural.—Distillation with 12 per cent. hydrochloric acid, directly according to Tollens (Browne, *Handbook of Sugar Analysis*, 1912, 450) and with steam according to Jolles (*Sitzungsber. Akad. Wiss. Wien* 1905, **114** (**IIB**), 1191) respectively were tried. Steam distillation using phosphoric acid (Youngberg, *Bioι. Chem.*, 1927, **73**; 599) was also attempted. It was observed that furfural was not completely removed by steam even after eight hours of distillation. The collected distillates, which were never less than three litres, rendered the adoption of gravimetric methods impracticable,

Methods involving the use of (1) phloroglucinol (Kröber, *J. Landw.* 1900, **48**, 357), (2) barbituric acid (Jäger and Unger, *Ber.*, 1902, **35**, 4440; 1903, **36**, 1222), (3) bisulphite-iodine (Jolles, *loc. cit.*), (4) electrical titration (Pervier and Gartner, *J. Ind. Eng. Chem.* 1923, **15**, 1167, 1255), (5) excess of bromine (Powell and Whittekar, *J. S. C. I.*, 1924, **43**, 35T), (6) phenylhydrazine (Ling and Nanji, *Biochem. J.*; **15**, 466), (7) aniline acetate (Youngberg, *J. Biol. Chem.*, 1927, **73**, 599) and (8) benzidine reagent (McCance, *Biochem. J.*, 1926, **20**, 1111) were tried.

The first two were gravimetric, the last two colorimetric and the rest volumetric. The colorimetric methods could not be applied to the hydrochloric acid distillates owing to difficulties in matching colours.

Furfural.—Known quantities of pure furfural were estimated according to the different methods (Table I).

TABLE I.

Method	Furfural		Deviation per cent.
	Actual, grams	As estimated, grams	
Phloroglucinol	0·1202	0·1245	+ 3·6
Barbituric acid	,,	0·1205	+ 0·3
Bisulphite-iodine	,,	0·1195	+ 0·7
Elecrical titration...	0·0992	0·0996	+ 0·4
Excess of bromine...	0·1290	0·1285	+ 0·3
Phenylhydrazine	0·2362	0·2369	+ 0·3

It was found difficult to determine the end-point by the electrical titration method. The bromine method was easy to conduct and gave quite consistent results.

Samples of pure xylose were treated with 12 per cent. hydrochloric acid and distilled directly according to Tollens (*loc. cit.*) and with steam according to Jolles (*loc. cit.*), the results being given in Tables II and III, respectively.

TABLE II.

Method	Phloroglucinol	Barbituric acid	Bisulphite	Bromine	Phenyl-hydrazine
Deviation per cent. from the actual	+ 2·8	—2·4	2·5	—0·1	—1·4

TABLE III.

Method	Bisulphite	Bromine
Deviation per cent. from the actual ...	4·5	—0·2

Similar specimens steam distilled with 85 per cent. phosphoric acid and estimated colorimetrically gave results differing on an average by 0·4 per cent. from the actual.

The following results (Table IV) were obtained for pure arabinose distilled with 12 per cent. hydrochloric acid according to Tollens.

TABLE IV.

Method	Phloroglucinol	Barbituric acid	Bisulphite	Bromine	Aniline acetate
Deviation per cent. from the actual.	− 1·1	− 2·0	+ 2·5	+ 0·2	Very large

It was inferred from the above that the bromine method of Powell and Whittekar gave the most accurate results.

It was found, on further trial, that the experimental error arising through the use of the same method could be reduced to a minimum by (*a*) using ground glass joints in place of rubber or cork stoppers for distillation of furfural and (*b*) titrating, after completion of reaction, against N/50 thiosulphate. The deviations being small and on either side of the mean, it was possible to reduce the error, still farther, by repetition.

SUMMARY.

Distillation with 12 per cent. hydrochloric acid from an all glass apparatus followed by estimation of furfural in the distillate by the bromine method of Powell and Whittekar was found to be the most satisfactory technique for estimating pentoses and pentosans.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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CONTRIBUTIONS TO THE STUDY OF SPIKE-DISEASE OF SANDAL (*SANTALUM ALBUM*, LINN.).

PART XI. New Methods of Disease Transmission and their Significance.

By M. Sreenivasaya.

In a previous communication (*J. Indian Inst. Sci.*, 1928, **11A**. 244), it has been shown that spike can be transmitted to healthy sandals by budding. The process is, however, rather a troublesome one to conduct and the percentage of buds which grow is very low. Further experiments have now led to the discovery that the disease may be transmitted with much greater certainty by the process of 'patch-grafting' and still more readily by 'leaf insertion.' The latter operation is particularly effective as well as simple to conduct and promises to be of very great value in future investigations.

I. *Transmission by 'Patch-grafting.'*—In the course of several buddings carried out in the laboratory, it was found that some of the stocks operated upon succumbed to the disease although the bud did not grow. On examination, the patch was found to have remained green and to have become intimately fused with the stock, so that it was evidently responsible for transmitting the infection. This observation suggested that an ordinary patch of bark tissue including the cortex and the bast which can easily be skinned from a scion, might, on grafting, induce the disease in healthy stocks. A number of healthy sandals were subjected to this 'patch-grafting' employing three kinds of patches, (1) those with active buds, (2) those with dormant buds and (3) those without buds.

It will be seen from table I, that patches bearing actively growing buds are more virulent and transmit the disease to healthy stocks in a much shorter time than those with dormant buds. Patches completely free from buds are less infective than the other two varieties as shewn by the longer periods taken by the bud-free patches to induce the disease. This confirms the view generally held that young and actively growing tissues are most favourable for the multiplication of the virus.

TABLE I.

Results of patch-grafting under controlled laboratory conditions.

(Sandals nurtured by *Acacia farnesiana* were used as stocks for all the experiments.)

Infective material	Number of stocks treated.	Percentage of fusions	Percentage of transmissions	Minimum period for manifestation of disease symptoms (days)
Active buds ...	29	88·0*	27·5	57
Bud-bearing patches.	35	93·5	11·4	98
Bud-free patches ...	33	9·6	9·1	158

The method of patch-grafting was also tested as regards its capability for inducing disease in stocks growing under sylvicultural conditions. The operation was conducted at two places representing two distinct types of forest. The results are summarised in table II.

TABLE II.

Results of patch-grafting under sylvicultural conditions in Coorg and North Salem.

Area	Kind of Patch-graft	Number of stocks treated	Precentage of fusions	Percentage of disease transmission within 120 days
Coorg	**Healthy** ...	34	97	6
	Spike ...	39	95	44
North Salem	**Healthy** ...	19	100	10
	Spike ...	20	90	65

60 trees in Coorg and 39 in North Salem were kept under observation as controls. 10 per cent. of the former and 5 per cent. of the latter became infected from natural causes during the above-mentioned period.

It is clear that a remarkably high percentage of successful fusions can be obtained by this method of disease transmission even in

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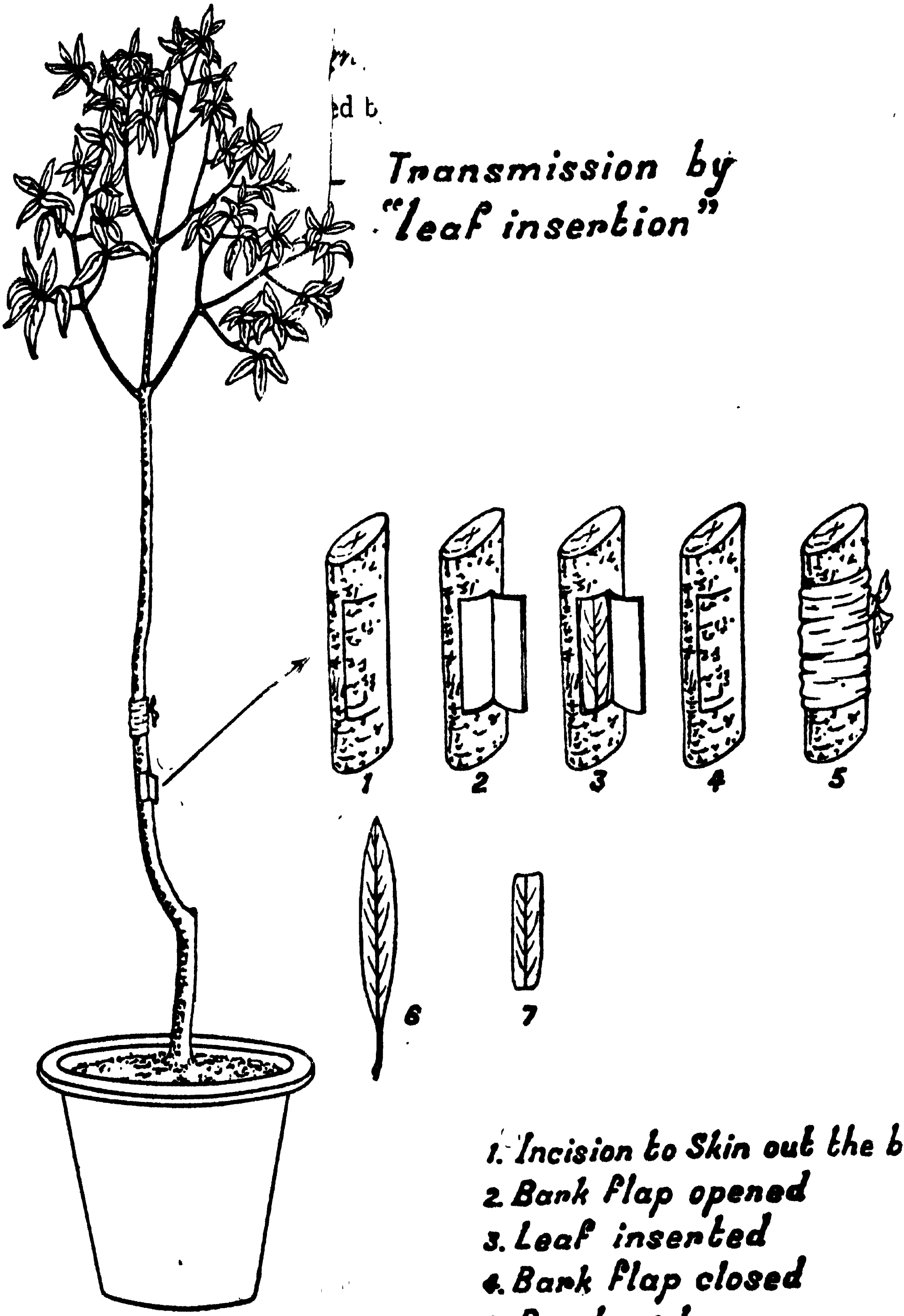
* The bud grew only in 24 per cent. of these cases.

ed b

Transmission by "leaf insertion"



1. Incision to skin out the bark
2. Bark flap opened
3. Leaf inserted
4. Bark flap closed
5. Bandaged
6. Spike leaf
7. Spike leaf trimmed for inserti

sylvicultural conditions which would ordinarily prove fatal to scion grafting and budding operations. The higher percentage of disease transmissions obtained in the North Salem area indicates that the trees in that region are highly susceptible to disease. Sandals in the Coorg area are less susceptible, while the pot cultured plants in the laboratory exhibit a high degree of resistance as shown by table I.

The advantages claimed for this new method of disease transmission are:—(1) the operation is extremely simple, in marked contrast to the delicate and difficult technique of budding; (2) the method lends itself to quantitative treatment as the infective patches can be either measured or weighed; (3) weather conditions do not affect the success of the operation; high percentage transmissions have been obtained in the hottest months of the year; (4) the operation can be successfully applied to infect stocks growing under sylvicultural conditions; (5) there is no need for the buds to burst from the scion which is essential for a successful budding operation; and (6) controls can be run with uninfected patches.

II. *Transmission by 'leaf insertion'.*—It has been shown that the biochemical changes occurring in the plant with the onset of disease, manifest themselves in the leaf tissue in a more intensive and pronounced manner than in any other part of the plant. It appeared likely therefore, that the diseased leaf might be a seat of high virus concentration. In order to test this hypothesis fresh diseased leaves trimmed to the form of a rectangle, were inserted between the wood and the bark of the stem (see plate I) covered with the bark flap and bandaged with wax cloth. If the operation was successful, the injured portion fused by callous formation in the course of 10 to 15 days and the leaf tissue became completely covered.

Under normal conditions, it was found that the disease manifested itself in the course of 45 to 60 days but the period varied widely with the number of leaves inserted, the size of the stock and the individuality of the plant.

Of the fourteen stocks operated upon by this method, ten, or 72 per cent., have already succumbed to the disease, a very high proportion for pot-cultured stocks which, as already pointed out, are more resistant than those growing under natural conditions. This supports the theory of the high concentration of the infective principle in the leaf.

Leaf insertion thus affords a very convenient method of disease transmission. The operation is simple and a large number of leaves can be inserted without seriously injuring the stock, while the percentage of effective transmissions is very high and the period of infection can be reduced by increasing the number of leaf insertions. The method lends itself to quantitative control because the leaves can be weighed and their areas measured. Moreover, controls may be run by inserting healthy leaves.

III. TRANSMISSION BY OTHER METHODS

(*a*) *Patch-grafting of root bark on root.*—The tissue fuses with the stock quite readily but up till now (16 months after the grafting operation), no positive results have been obtained. This indicates that either the root bark does not contain the infective principle or the root is not a region susceptible to infection.

(*b*) *Leaf mutilation.*—Diseased leaves were put on the healthy leaves of a healthy stock and crushed together so as to ensure an exchange of sap between them. No disease transmission has resulted during a period of 19 months. This points to the conclusion that the leaf is not a vulnerable region and confirms the observation that with an abundance of green foliage, an infected stock tends to resist the disease. (*Cf.* Sreenivasaya, *Proc. Indian Science Congress*, *Section of Botany*, 1930.)

(*c*) *Wood grafting.*—Wood from young diseased scions was taken after removing the bark and carefully inserted into the healthy sandal in place of a corresponding piece which was cut from the stock. The bark flap was then bandaged with wax cloth. Fusion of the bark took place in the course of three weeks.

The operation is a difficult one to conduct but in no case in which it was successful has the disease been transmitted, although the experiments were started in January 1928. The wood tissue consequently does not appear to be infectious, particularly as the corresponding bark tissue produced symptoms of the disease in another batch of stocks in the course of four months.

(*d*) *Transmission by injection of tissue fluids.*—Attempts to induce the disease by injecting stocks with the tissue fluids derived from various kinds of diseased tissues were not successful. Difficulty was found in injecting the juice into young, healthy, pot-cultured stocks as the tissues are too hard to pierce with a hypodermic needle. The method finally adopted was to insert filter papers freely impregnated with the tissue fluid under the bark in the same way as a leaf in the operation of patch-grafting. The fusions were successful but symptoms of disease have not appeared on any of the stocks which were operated upon in June 1929. The tissue fluids from the leaf, bark and root have been tried but in no case there has been any disease transmission.

The infective principle may become neutralised by other cell contents or rendered innocuous in some way during the operation of extraction. Support is lent to this view by the fact that if the leaf tissue is inserted after fine pulping, it does not transmit the disease so that the mere disintegration of the cells appears to lead to a destruction or neutralisation of the infective agent. Attempts are now being made to isolate the virus without damaging the leaf by methods involving adsorption and cataphoresis.

In the course of these experiments, indications have been obtained that several varieties of sandal exist, differing in their disease-resisting properties. In particular, one very resistant variety appears to be characterised morphologically by a definitely ovate leaf, a stem rich in lenticels and a root with high haustorising capacity. Attempts are now being made to classify other varieties.

SUMMARY.

Several new methods of disease transmission have been described, of which 'patch-grafting' and 'leaf insertion' are the most successful.

Although the transmission of disease has not been so far secured by the other methods, they have led to important conclusions as to the distribution of the infective principle in the plant and the susceptibility to infection of certain tissues in different regions of the plant.

The experiments strongly support the view that laboratory pot-cultured plants are more resistant to disease, than those growing under natural conditions.

Some evidence has been obtained regarding the existence of varieties of sandal differing in their disease resisting properties.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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I.—THE PHYSICAL PROPERTIES OF PURE TRIGLYCERIDES.

*By R. B. Joglekar and H. E. Watson.**

Since the publication of Scheij's exhaustive paper on the physical properties of the fatty acids and the triglycerides (*Rec. trav. chim.*, 1899, **18**, 169), various more or less detached observations have been made in which the values recorded do not always agree with this author's figures. As a part of the general scheme of work on oils and fats and their derivatives which is being conducted in these laboratories, it seemed desirable to revise this branch of the subject and simultaneously to determine additional physical properties. The principal results together with some data on binary mixtures are given in the present paper.

EXPERIMENTAL.

The acids used for preparing the glycerides were the purest commercially obtainable. Stearic and palmitic acids were crystallized from 95 per cent. alcohol and myristic and lauric acids from 75 per cent. alcohol until three consecutive crystallizations gave a product with the same solidifying point. The acids formed large crystals and appeared to be very pure. The molecular weights agreed with the calculated values. The solidifying points and refractive indices are given in Table I and compared with those of de Visser (*Rec. trav. chim.*, 1898, **17**, 182), Scheij (*loc. cit.*) and Pascal (*Bull. Soc. chim.*, 1914, [iv], **15**, 360).

TABLE I.

Solidifying Points and Refractive Indices of Acids.

Acid		Lauric	Myristic	Palmitic	Stearic
Solidifying point ...	de Visser ...	...	...	62·6°	69·32°
	Scheij ...	43·4°	53·8°	62·6°	69·3°
	Joglekar and Watson.	43·4°	53·8°	62·2°	69·35°
		60°	60°	80°	80°
Refractive index ...	Scheij ...	1·4266	1·4307	1·4269	1·4300
	Pascal ...	...	...	1·4269	1·4298
	Joglekar and Watson.	1·4264	1·4305	1·4266	1·4296

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* *Reprinted with additions from Journal of the Society of Chemical Industry*, 1928, 47, 365 T.

The low solidifying point of palmitic acid is noticeable. The same value was obtained from three different original samples after 12 crystallizations from 95 per cent. alcohol.

Two methods were adopted for preparing the triglycerides, the first a modification of Bellucci's process (*Atti. R. Accad. Lincei*, 1911, **20**, i, 125). Three molecules of glycerin and one of the acid were heated to 180° and the temperature slowly raised to 215° during two hours at 30–40 mm. pressure; the temperature was then raised to 250° in one hour and maintained at that point for one hour, the pressure being reduced to 6 mm. The second method was that of Partheil and Velson (*Arch. Pharm.*, 1900, **238**, 261), using tribromhydrin and the silver salt of the acid. The first method gave a larger yield of a purer product but after final purification, the glycerides obtained by the two processes had identical physical properties.

The crude product was crystallized six or seven times from concentrated alcohol in which the triglycerides are sparingly soluble and recrystallized from ligroin and ether. In the cases of palmitin and stearin, acetone and chloroform were subsequently used as solvents, but no further change in physical properties was brought about. The last traces of solvent were removed by heating for 2 to 3 hours at 100° under reduced pressure.

PHYSICAL PROPERTIES.

An examination of the physical properties of several of the substances during the course of their purification showed that the melting points and refractive indices afforded little criterion as to purity, but the densities, viscosities and solidification points varied appreciably even when a fair degree of purity had been attained. For example, two samples of tristearin with m.p. 71·7° and refractive index 1·4395 did not change these values after repeated purification. The densities which were 0·8622 and 0·8619 changed to 0·8631, the viscometer flow-times of 582·5 and 577·8 seconds changed to 591·4 and the solidifying points 69·4° and 69·6° to 71·3°. The viscosity is thus, perhaps, the most sensitive indication of impurity, but its determination requires a very careful temperature adjustment and for practical testing the solidifying point is nearly as accurate and much easier to measure. The solidification of the triglycerides is, however, as is well known, of a complex nature, and the determination of the solidifying point requires certain precautions. On heating a crystallized sample of, say, tristearin, melting occurs at 71·7°, but if the sample has been previously melted and suddenly cooled, it first melts at about 55°, then solidifies and again melts at 71·7°. This double melting point has formed the subject of several investigations (Vandevyer, *Ann. Chim. analyt.*, 1900, **5**, 321; Guth, *Z. Biol.*, 1902, **44**, 78; Kremann, *Monatsh.*, 1912, **33**, 1063; Bokhörst, *Diss. Amsterdam*, 1916; Nicolet, *Ind. Eng. Chem.*, 1920, **12**, 761; Grün, *Ber.*, 1921, **54**, 290; Klimont, *Oesterr. Chem.-Ztg.*, 1922, **25**, 22), but no very definite conclusions regarding the composition of the two modifications appear to have been reached. Additional data have been collected during the present series of experiments, and this aspect of the subject is still under investigation. For practical purposes it is sufficient to regard the glycerides as existing in two forms, the α-form obtained by crystallization from solvents and the β-form by heating the α-form to its melting point which appears to be a transition temperature. On cooling, crystallization does not occur at all readily and in some cases, the transformation is so slow that the liquid appears to be the stable form. In order to examine this point more closely, a sample of tristearin melting at 71·8° was divided into 3 portions of about 1 g. each and placed in covered glass dishes. Two of these were heated until the glyceride was completely melted, and all three were then placed in an oven at 65°. The solid did not melt even on prolonged heating but the liquid began to crystallize in about half-an-hour, crystallization starting usually from one point. If the liquid was seeded with a crystal of the α-form, crystallization started at once; but the rate of growth was very slow, some 10–15 minutes being required for complete solidification. To ensure that the initial crystallization was not due to accidental contamination, some of the substance was filtered into glass tubes which were sealed and then placed in boiling water for some time. The onset of crystallization was delayed but not inhibited, solidification occurring in 2 to 3 hours at 65°.

It is conceivable that the change of form may take place in one of two ways; either crystals of the α-form may grow directly from the liquified β-form or, less probably, the β-form may change to the α-form in a super-cooled liquid state, slow crystallization ensuing subsequently. That the latter hypothesis is untenable was shown by melting the glyceride in sealed tubes and maintaining the liquid at 65° during two hours. The tubes were then chilled in cold water. The solid melted at 55° the melting point of the β-form, but resolidified in a few moments showing that numerous nuclei of the α-form had been produced by chilling. This formation of nuclei is, no doubt, responsible for the change of the β into the α-form in the solid state at room temperature frequently observed during the experiments. When it takes place there is a remarkable swelling into sponge-like formations, and the containing vessel is often broken.

It appears probable that what we have termed the α and β-forms are not single substances but mixtures of two or more varieties in internal equilibrium. This is almost certainly so in the case of the β-form after solidification. More detailed knowledge is not, however, necessary for the determination of the solidifying point as a physical constant. What is essential is a recognition of the fact that the transformation from β to α requires a considerable time and the heat evolved is not very great. Consequently, if the quantity of substance is too small or the jacket round the tube is at too low a temperature, low values are obtained for the solidifying point. It was found that consistent values can be obtained if 3 g. or more of the glyceride are used in a 2 cm. tube surrounded by an air jacket which, in turn, is immersed in a bath the temperature of which is raised during the experiment so as to be not more than 4° below that of the substance. Stirring has no effect in the case of tristearin, but it should be adopted with the lower members as it decreases the time required for the temperature rise. Owing to transformation taking place, the lower solidification points could not be definitely measured but only showed as slight breaks in the time-temperature curve. As the substance frequently did not melt completely, the melting points for the β-form were taken as the temperature of initial softening in a capillary tube on raising the temperature about 1° per minute. The melting points for the α-form are the temperatures of complete liquefaction under similar conditions.

Refractive indices were determined with an Abbé refractometer checked against a standard piece of fluorite. As has been remarked previously (*J. Indian Inst. Sci.*, 1922, **5**, 50), the use of this instrument at high temperatures is attended by some uncertainty as regards the temperature of the prisms. Most of the present measurements were made with heating water flowing at a definite rate and the temperature taken as the mean of the inlet and outlet temperatures. A small correction was subsequently applied by circulating the water very rapidly with a pump so that the temperature drop in passing through the prism heaters was less than 0·1° at 80°. The exposed prism face was covered with a plug of cotton wool except just at the moment of reading and the prism temperature assumed to be the same as that of the water, a correction being applied for the emergent stem. Scheij when measuring the refractive index of water at 80° obtained values 4 units in the fourth place of decimals lower than the accepted values, owing to the change in refractive index of his prism. A similar experiment with the Abbé refractometer used in the present experiments, gave correct values and so no correction has been applied.

The bath used for measurements of density, viscosity and surface tension, consisted of a 2 litre beaker of water heated electrically and fitted with a stirrer, standing in another beaker as an air jacket and carefully covered. With a steady source of heating current and hand regulation, the temperature remained constant to 0·01° and could be readily varied through a wide range.

Densities were determined in a 5 cc. pyknometer with two fine capillary limbs calibrated with water at each temperature employed. The values obtained are given in Table II.

TABLE II.

Densities of triglycerides at different temperatures.

Temp.	Caprin.	Laurin.	Myristin.	Palmitin.	Stearin.
40°	0·9204	...	...	...	...
60°	0·9059	0·8943	0·8860	...	...
70°	0·8986	0·8872	0·8790	0·8730	...
80°	0·8913	0·8801	0·8722	0·8663	0·8632
Mean change per 10°	0·0073	0·0071	0·0069	0·0067	0·0067

Viscosities were measured in a modified Ostwald viscometer with a trap in the upper bulb to enable a constant volume of liquid at the required temperature to be taken. This was calibrated at 30° with solutions of glycerin in water varying in concentration from 59 to 62 per cent., Muller's (*Wien. Ber.*, 1924, **133**, 133) figure 0·0857 for the viscosity of a 61·44 per cent. solution having $d^{30°}$ 1.1517 being taken as standard. A small correction was applied for the expansion of the glass at the higher temperatures. Table III shows the values obtained.

TABLE III.

Viscosities of triglycerides at different temperatures.

Temp.	Caprin.	Laurin.	Myristin	Palmitin.	Stearin.
40°	0·1879	...	...	...	...
45°	0·1006	0·2198	...	...	...
60°	0·0777	0·1359	0·1771	...	...
70°	0·0688	0·1030	0·1342	0·1679	...
75°	0·0625	0·0911	0·1170	0·1467	0·1850
80°	...	0·0809	0·1035	0·1292	0·1621
85°	0·0551	0·0722	0·0920	0·1144	0·1431

Surface tension was determined by the maximum bubble pressure method (*cf.* Sugden, *J.C.S.* 1924, **125**, 27, etc.), the apparatus being standardized with carefully purified benzene. The surface tension of this was assumed to be 27.58 at 30°. The results are given in Table IV.

TABLE IV.

Surface tension of triglycerides (dynes/sq. cm.) at different temperatures.

Temp.	Caprin	Laurin	Myristin	Palmitin	Stearin
40°	28·7	...	...	...	...
60°	27·3	27·9	28·7	...	...
70°	26·6	27·3	27·7	28·3	...
80°	25·9	26·6	27·2	27·6	28·1
P	1404	1648	1892	2134	2376
P (calc.)	1399	1633	1867	2101	2335
diff.	5	15	25	33	41

The parachors $P = M\gamma^{1/4}/d$ (*cf.* Sugden, *J.C.S.*, 1924, **125**, 1184) have been calculated from the surface tensions at 80° and form a very uniform series, the mean difference for C_2H_4 being 81. There is however a marked difference from the values calculated with Sugden's data according to which the difference for C_2H_4 is 78. Taking Walden's values for density and surface tension at 80° (*Z. physikal Chem.*, 1910, **75**, 560) the parachors for tripalmitin and tristearin are 2120 and 2360. Sugden gives the values from the same data as 2252 and 2380, the former value being due to an error in the formula assumed for tripalmitin.

A summary of the above results, with figures for the melting and solidifying points and values given by other observers is shown in Table V.

Scheij's values are thus mainly confirmed, the chief differences being in the densities of palmitin and stearin. These were determined in an air-bath by Scheij, and it is possible that the temperature of the glyceride was not exactly that of the bath. With regard to the lower melting points of laurin and myristin, the same author mentions that laurin softened at 44° and myristin between 35° and 45°, but does not state that these are the lowest temperatures at which softening was observed. The densities calculated from Jæger and Kahn's figures

TABLE V.

Physical properties of triglycerides.

—	Author	Caprin	Laurin	Myristin	Palmitin	Stearin
Melting point (1)	B[1].	...	...	...	61	71·0
	S[2].	31·1	46·2	56·5	65·1	71·6
	G[3].	...	...	...	65·5	71·5
	J. & W.	31·6	46·2	56·5	65·6	71·8
Melting point (2)	B.	...	...	...	..	55·0
	S.	...	44	35	45	55
	J. & W.	...	18·0	33·0	46·2	55·0
Solidifying point	K[4].	...	...	...	62·6	56·0
	N[5].	...	...	...	...	69·0
	J. & W.	30·3	45·3	56·1	65·2	71·3
Refractive index	S.	1·43697 (60°)	1·44039 (60°)	1·44285 (60°)	1·43807 (80°)	1·43987 (80°)
	P. & N[6].	...	...	...	1·4371 ,,	1·4396 ,,
	P[7].	...	...	...	1·4377 ,,	1·4385 ,,
	J. & W.	1·4370 ,,	1·4402 ,,	1·4428 ,,	1·4376 ,,	1·4395 ,,
Density	J. & K[8].	0·9056 ,,	0·8945 ,,	...	0·8671 ,,	0·8672 ,,
	S.	0·9057 ,,	0·8944 ,,	0·8848 ,,	0·8657 ,,	0·8621 ,,
	W[9].	...	...	...	0·8666 ,,	0·8624 ,,
	J. & W.	0·9059 ,,	0·8943 ,,	0·8860 ,,	0·8663 ,,	0·8632 ,,
Surface tension	W.	...	...	...	26·87 ,,	27·21 ,,
	J. & W.	27·3 ,,	27·9 ,,	28·7 ,,	27·6 ,,	28·1 ,,
Viscosity	W.	...	...	...	0·1456 (70°)	0·1741 (70°)
	J. & W.	0·0688 (70°)	0·1030 (70°)	0·1342 (70°)	0·1679 ,,	0·1850 (75°)

[1] Berthelot. *Ann. Chim. Phys.* 1854, (3) **41**, 240.

[2] Scheij. *loc. cit.*

[3] Guth. *Z. Biol.*, 1902, **44**, 78.

[4] Kremann, *Monatsh.* 1912, **33**, 1063.

[5] Nicolet, *Ind. Eng. Chem.*, 1920, **12**, 761.

[6] Partheil and Nelson, *Arch. Pharm.*, 1900, **238**, 261.

[7] Pascal, *Bull. Soc. chim.*, 1914, **15**, 360.

[8] Jæger and Kahn, *Proc. K. Akad. Wentensch. Amsterdam*, 1915, **18**, 285.

[9] Walden, *loc. cit.*

agree for the two lower glycerides but appear distinctly high for the upper members of the series. Details as to the purity of their compounds are not available. Walden's values for density agree fairly closely, but his figures for viscosity and surface tension are much lower than those we have obtained. The melting points of his glycerides were 65·0° and 71·2° (unc.) so that they do not appear to have been as pure as our samples and this would account for his low results.

BINARY MIXTURES.

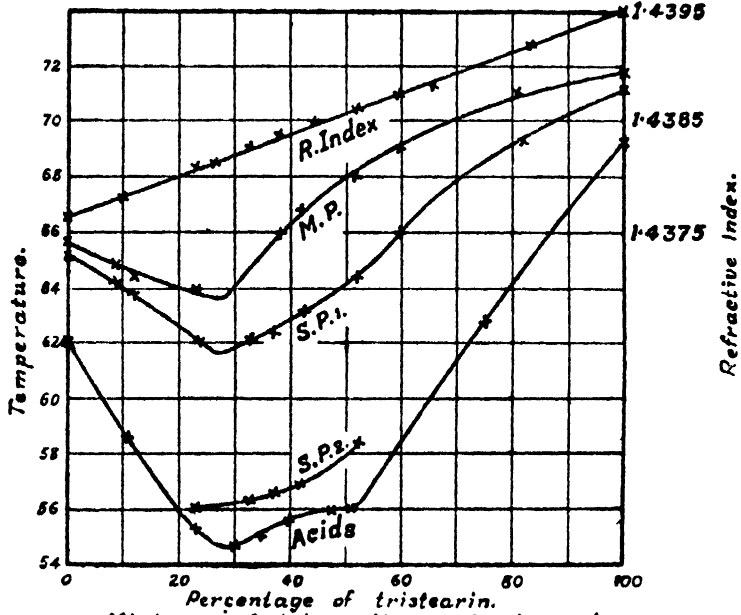
The solidification points of mixtures of tripalmitin and tristearin were determined hy Kremann (*loc. cit.*) with the object of ascertaining if there was a flat portion on the curve as there is in the case of palmitic and stearic acids. His results appear to refer to mixtures of tripalmitin with the two modifications of tristearin in indefinite proportions, the low melting variety predominating.

We have succeeded in obtaining figures which appear to be characteristic of the mixture of the high melting modifications and also a few points for which at least one constituent is in the β-form. Some care seems to be necessary in preparing the mixtures as the glycerides do not mix readily, and it was found advisable to shake the molten mixtures in a stoppered bottle.

With mixtures containing from 25 to 50 per cent. of tristearin, if the molten mass is cooled until solidification takes place and is then vigorously stirred, definite solidification points in the neighbourhood of 58° are obtained. If, on the other hand, the liquid is kept at 56° for some minutes before stirring and not allowed to cool below this temperature, much higher solidification points are found. Double solidification points could not be obtained for the other mixtures. Fig. 1 shows the results graphically and it may be seen that the curve is characteristic of a simple mixture and does not resemble the one for the corresponding acids which is given in the same diagram. The latter was determined in the same apparatus and with the acids from which the glycerides were prepared. The figures are slightly lower than those given by de Visser (*loc. cit.*) owing to the lower solidification point of the palmitic acid used.

The refractive indices of mixtures of tripalmitin and tristearin were measured by Pascal (*loc. cit.*) who found a minimum in the curve at 80° and a maximum at 60°, while at 70°, the relation was approximately linear. The refractive index of the tristearin used by this author was only 1·4385 at 80°, considerably lower than the value obtained by other observers, and although the preparation is described as synthetic, no details are given, and its purity must be regarded as doubtful. We have been unable to confirm Pascal's values, but find a relation between refractive index and composition which is linear within the limit of experimental error both at 70° and 80°. The results at 80° are shown in Fig. I. The figures for 70° are 0·0036 higher. Measurements could not be made at 60° owing to solidification.

Fig I



Mixtures of tripalmitin and tristearin.

Determinations of the density, viscosity and surface tension of palmitin-stearin mixtures have also been made, but these present no unusual features. The solidifying points of caprin-stearin mixtures have been investigated and exhibit certain peculiarities which will be dealt with in detail on another occasion.

Department of General Chemistry,
Indian Institute of Science,
Bangalore.

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II.—THE PREPARATION AND PHYSICAL PROPERTIES OF α-MONOGLYCERIDES.

By R. S. Rewadikar and H. E. Watson.

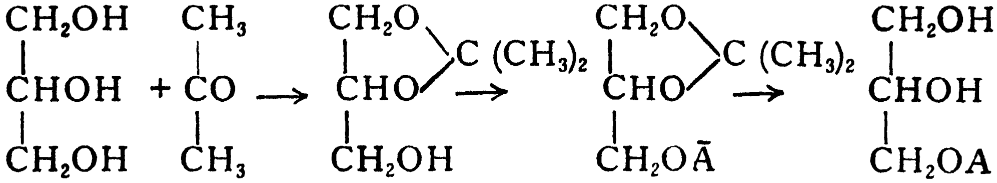
Simultaneously with the investigation of the triglycerides outlined in the previous paper, experiments of a similar nature were conducted with the α-monoglycerides, the literature of which is even more scanty than that of the triglycerides. The glycerides of acids with an even number of carbon atoms from lauric to stearic have been previously prepared, but in most cases, the melting point is the only physical constant which has been measured and the figures given by different authors vary considerably as shown in Table I.

TABLE I.

Melting Points of α-Monoglycerides.

...	Berthelot.	Hundes-hagen.	Smith.	Thieme.	Guth.	Krafft.	Grün.	Fischer.	Brash.	Adam, Berry and Turner.
Laurin ...	...	...	...	58·9	...	59	61	62–63	...	...
Myristin ...	...	...	...	...	...	68	69	...	...	70-71
Palmitin ...	58	...	63	...	65	72	74	78–79	68	76–77
Stearin ...	61	60–62	...	...	73	78	80	81–82	...	...

Berthelot (*Compt. rend.*, 1853, **37**, 398) was the first to attempt the synthesis of the monoglycerides from the acids and glycerin. His work was repeated more systematically by Chittenden and Smith (*Am. Chem. J.*, 1885, **6**, 225) and Belucci (*Gazzetta*, 1912, **42**, 283), but the products obtained were found to be mixtures. Guth (*Z. Biol.*, 1902, **44**, 78), Krafft, (*Ber.*, 1903, **36**, 4342) and Grün (*Ber.*, 1910, **43**, 1288; 1912, **45**, 3421) used the chlorhydrins as starting materials by allowing them to react with the sodium or potassium salts of the acids or by esterifying a di-halogen hydrin and then converting the halogens into hydroxyl groups. Thieme (*Ber.*, 1913, **46**, 1653) pointed out that reactions of this type were not simple, but yielded other products as well as those desired, and Fischer (*Ber.*, 1920, **53**, 1589, 1621) showed that the acid radicle might change its position during the course of the reaction so that the constitution of the glyceride produced in this way could not be definitely established, Abderhalden (*Ber.*, 1914, **47**, 2888) appears to have been the first to prepare α-monoglycerides of definite constitution. He isolated the optically active epihydrin alcohol and esterified the lower members of the fatty acids, with it. The products were purified by distillation in vacuo. Fischer and his collaborators (*Ber.*, 1920, **53**, 1589) achieved the same result by using as starting material isopropylidene glycerin, the constitution of which had been definitely established by Irvine, Macdonald and Soutar (*J.C.S.*, 1915, **107**, 342). This was condensed with fatty acid chloride and the isopropylidene linking removed, giving the α-glyceride.



The glycerides could be readily condensed with acetone giving the intermediate isopropylidene glyceride thus showing that the acid radicle had not changed place on hydrolysis, since acetone will only condense with two adjacent hydroxyl groups. (*Ber.*, 1920, **53**, 1606.)

Fischer (*Ber.*, 1920, **53**, 1640) also prepared the α-monoglycerides by the alcoholysis of alkyl esters of fatty acids with glycerin and by the degradation of triglycerides using excess of glycerine. Bergmann and Sabety (*Z. physiol. Chem.* 1924, **137**, 47) prepared optically active α-monolaurin from 2-phenyl 5-chlormethyl oxazolidine. While the present experiments were in progress, Brash (*J. Soc. Chem. Ind.*, 1927, **46**, 481 T) prepared α-monopalmitin by a method which he considered superior to that of Fischer, *viz.*, by heating the lead salt of the fatty acid with α-monochlorhydrin. His palmitic acid was admittedly impure and this was no doubt a contributory cause to the low melting point (68°) of his product. Still more recently Adam, Berry and Turner (*Proc. Roy. Soc. A.* 1928, **117**, 540) have prepared monomyristin and palmitin by Fischer's method. The only property recorded was the melting point as given in Table I.

We have now prepared in a pure state the α-monoglycerides of acids with an even number of carbon atoms from C_8 to C_{18} and determined their melting points, refractive indices, densities, viscosities and surface tensions. As far as possible, alternative methods of preparation have been adopted in order to compare the products obtained in different ways.

It has been shown by Fischer (*loc. cit*) that on replacement of the iodine in α-iodo-$\beta\gamma$-acylated glycerin by hydroxyl, the $\alpha\gamma$-acylated glycerin results. It appeared of interest to see if a similar change would take place with a di-halogen compound and consequently allyl laurate and stearate, and their dibromides were prepared. Attempts to prepare the monoglycerides by substituting hydroxyl groups for the bromine were not successful as the bromine could not be completely removed.

EXPERIMENTAL.

Isopropylidene glycerin was prepared by Fischer's method (*Ber.* 1920, **53**, 1607) and obtained in 70 per cent. yield; b. p. 79°/9 mm.

α-monostearin.—The stearic acid used for this preparation was crystallized 3 times from 90 per cent. alcohol and melted at 69·3°. Its equivalent weight agreed with theory.

(*a*) Stearyl chloride was condensed with isoproplylidene glycerin dissolved in dry quinoline by standing at room temperature (25°) for 48 hours according to Fischer's method. The yield of acetone compound was 80 per cent. of the theoretical value and the crude product was used as such, since recrystallization seemed liable to produce hydrolysis. α-monostearin was obtained in 80 per cent. yield on hydrolysis with concentrated hydrochloric acid (1·19) at 20–25°. After washing with ether and crystallizing from the same solvent, the melting point was 82°, agreeing with Fischer's value.

(*b*) Methyl stearate m. p. 38·5–39° made from the stearic acid and methyl alcohol was heated with 4 mols. of glycerin containing 4 per cent. hydrochloric acid on the water-bath for various periods, moisture being excluded, but no alcoholysis took place.

(*c*) A mixture of methyl stearate (7g.) and glycerin (9·2g.) was dissolved in 8·7 g. of acetone containing 2 per cent. of hydrochloric acid and refluxed for 5 hours. The product was poured into water, and the solid which formed was recrystallized repeatedly from ether. 1 g. of a substance melting at 82° was obtained and the melting point did not change on mixing with the specimen obtained by method (*a*).

A similar experiment with 15 g. of methyl stearate in which the refluxing was continued for 48 hours, acetone removed by distillation and the residue dissolved in ether, yielded 2 g. of α-monostearin and 8 g. of a neutral product m. p. 40°–50° (probably crude isopropylidene monostearin which gave 3 g. of α-monostearin after hydrolysis and recrystallization from benzene.

(*d*) An attempt to obtain the glyceride by heating methyl stearate with isopropylidene glycerin in benzene solution containing hydrochloric acid was unsuccessful.

(*e*) Methyl stearate (10 g.) and anhydrous glycerin (9 g.) were dissolved in dry pyridine (50 cc.) and 1 cc. of a 7 per cent. solution of sodium methylate added. The mixture was heated for 24 hours on the water-bath, the pyridine distilled and the residue treated with a mixture of N/2 sulphuric acid and ether. 4·5 g. of α-monostearin with the same melting point as before were obtained.

(*f*) An alternative method of producing the glyceride was by oxidation of allyl stearate.

Allyl stearate was obtained in 80 per cent. yield by the action of allyl iodide on silver stearate suspended in benzene. It forms shining plates from methyl alcohol m. p. 35°. (Found: C, 77·6; H, 12·3. $C_{21}H_{40}O_2$ requires C, 77·7; H, 12·3).

Allyl stearate dibromide was prepared by adding a solution of bromine (1 mol.) in dry chloroform to a solution of allyl stearate (1 mol.) in the same solvent cooled to −3° with stirring. The reaction is very slow but the yield is quantitative. The substance forms a white solid from methyl alcohol; m. p. 45°. (Found : Br. 33·3. $C_{21}H_{40}O_2Br_2$ requires Br, 33·1.)

Oxidation of allyl stearate.—An attempt at oxidation with benzoylhydroperoxide was not successful, but a small yield of glyceride was obtained by permanganate oxidation in acetone solution at room temperature (30°). After a number of trials, the best yield (30 per cent.) was obtained as follows:—Allyl stearate (4 g.) was dissolved in acetone (700 cc.) containing 1·2 g. of magnesium sulphate at 2° (this amount of acetone is necessary to keep the ester in solution) and potassium permanganate (1·6 g.) equivalent to 1 atom of oxygen per 1 mol. of ester added slowly with stirring, the temperature being kept at 2°. The manganese dioxide was separated by filtration, suspended in water, dissolved by passing sulphur dioxide and the solution extracted with ether. This solution was washed with 10 per cent potassium bicarbonate solution and the ether distilled. The acetone solution was distilled and the residue taken up in ether. After recrystallization, the product melted at 82° and mixture with α-monostearin produced no change. (Found: C, 70·2 : H, 11·7. $C_{21}H_{42}O_4$ requires C. 70·4; H, 11·7.) Experiments with larger quantities of permanganate did not result in increased yield but caused the formation of stearyl glycollic acid previously obtained by Grün (*loc. cit.*) by the oxidation of α-monostearin.

α-monopalmitin.—The palmitic acid used for this preparation was a specially pure sample recrystallized 3 times from 90 per cent. alcohol. It had the correct equivalent weight and melted at 62·5°.

(*a*) A quantitative yield was obtained by condensing the acid chloride with isopropylidene glycerine. After recrystallizing from ether, the melting point was 76°.

(*b*) 5·4 g. of methyl palmitate gave by glycerolysis in pyridene solution (*cf.* α-monostearin (*e*)) 2·5 g. of glyceride with the same melting point. Glycerolysis in acetone solution gave a small yield.

(*c*) Equimolecular proportions of potassium palmitate and α-monochlorhydrin (b. p. 115°/7 mm.) were heated in a sealed tube at 180° for 6 hours and the product treated according to the method of Krafft. (*Ber.* 1903. **36**, 4342.) A very low yield of an impure product melting at 72–73° was obtained.

α-monomyristin.—Myristic acid was recrystallized from alcohol and melted at 53·6°.

(*a*) Myristyl chloride prepared from the above was redistilled and boiled at 169°/15 mm. A 75 per cent. yield of myristyl isopropylidene glycerin was obtained on condensation and a 90 per cent. yield of the glyceride on hydrolysis with hydrochloric acid (1·19) at –5°. The product was recrystallized from a mixture of ether and petroleum ether in which it is not very solúble and melted at 70°.

(*b*) 1 g. of the monoglyceride with the same melting point as the above was obtained from 7 g. of trimyristin and 2·8 g. of glycerin in a pyridine solution containing sodium methylate after standing for 3 days at room temperature (25°).

α-monolaurin melting at 63° was obtained in 90 per cent. yield by Fischer's method. The lauric acid was made from a pure specimen of methyl laurate prepared in the laboratory and melted at 43°. The acid chloride boiled at 123–124°/7 mm.

Allyl laurate.—Laurylchloride (13·2 g.) and quinoline (8 g.) were dissolved in dry chloroform (30 cc.). Allyl alcohol (3·5 g.) was gradually added at 0° while stirring and the mixture allowed to stand for 24 hours at room temperature. It was then extracted with chloroform and the solution shaken in succession with half-normal sulphuric acid, 10 per cent. potassium bicarbonate solution and distilled water. After drying with anhydrous magnesium sulphate and distilling the solvent, a neutral pale brown liquid (13·1 g.) was obtained which became semi-solid at -6°. The distillate was a colourless, mobile liquid boiling at 162–164°/20 mm.

Allyl laurate dibromide—Allyl laurate (9 g.) was dissolved in dry chloroform (50 cc.) and cooled in ice; bromine (6 g.) dissolved in the same solvent (10 cc.) was added while stirring and the reaction flask allowed to stand for 4 hours in ice. After removing the chloroform, the residue distilled at 220–222°/10 mm. as a pale yellow mobile liquid. (Found: Br. 39·3; $C_{15}H_{28}O_2Br_2$ requires 40·0.)

Attempts to prepare halogen hydrins from the above two compounds were not successful, inseparable mixtures only being obtained.

α-monocaprin.—Kahlbaum's synthetic capric acid boiling at 140–141°/6 mm. was used for this preparation. The acid chloride boiled at 97°/8 mm. The condensation of the acid chloride with isopropyliden glycerine was effected at -10° giving a 75 per cent. yield. Some difficulty was experienced in hydrolysing the acetone compound, but an 80 per cent. yield was finally obtained by treating an ethereal solution with 30 per cent. hydrochloric acid at -10° to -15° for 1 hour. The product after recrystallization from petroleum ether melted at 54°, and had saponification value 227·3 (calc. 228·1).

Diphenylurethane—α-monocaprin (1 mol.) was mixed with phenyl isocyanate (2 mols.) and the mixture allowed to stand during 48 hours at 25°. It was then made into a paste with carbon tetrachloride and filtered. The residue was dissolved in hot benzene and filtered and an 80 per cent. yield of a colourless substance melting at 101° obtained from the filtrate. (Found: C, 67·1; H, 7·4; $C_{27}H_{36}O_6N_2$ requires C, 66·9; H, 7·5.) The substance is therefore the diphenyl urethane of α-monocaprin.

Di-p-*nitrobenzoyl derivative.*—*p*-nitrobenzoyl chloride b. p. 156–157°/6 mm. was prepared from the acid and phosphorus pentachloride and a chloroform solution added to a solution of α-monocaprin in dry pyridine cooled to -10°. After standing for 3 days the pyridine was removed with acid and an almost theoretical yield of the mixed triglyceride obtained. Crystallised from hot alcohol it forms small pale yellow needles m. p. 94°. (Found: C, 59·4; H, 6·1, $C_{27}H_{32}O_{10}N_2$ requires C, 59·6; H, 5·9.)

α-monocaprylin was prepared from a sample of synthetic caprylic acid b. p. 115°/7 mm. the acid chloride of which boiled at 77°/8 mm. in the same way as α-monocaprin. The theoretical yield of an oil was obtained and this solidified on keeping in a vacuum desiccator for three days. On filtering at 0°, white shining plates m. p. 40° were separated and a further yield obtained by stirring the slightly acid filtrate with a little sodium carbonate and extracting with ether. The melting point was not raised on recrystallizing from petroleum ether. The saponification value was 257 agreeing with theory.

The *diphenyl urethane* was obtained in 80 per cent. yield in the same way as the caprin derivative. It forms a fine colourless powder from benzene m. p. 114°. (Found: C, 65·9; H, 7·2; $C_{25}H_{32}O_6N_2$ requires C, 65·8; H, 7·0.)

The *di*-p-*nitrobenzoyl derivative* was prepared in 80 per cent. yield in the same way as the caprin derivative. It forms pale yellow needles from alcohol m. p. 91·5°. (Found: C, 58·9; H, 5·6; $C_{25}H_{28}O_{10}N_2$ requires C, 60·0; H, 5·6.)

PHYSICAL PROPERTIES.

Melting points were determined in a capillary tube with a standard thermometer and corrected for exposed stem.

Refractive indices were measured on an Abbé refractometer at 93·1°, 85·7°, 78·7° and 65·0° except those of palmitin and stearin which could not be determined at the lowest temperature.

Densities were determined at the boiling point of water (97·3°) in a pyknometer consisting of a glass bulb of approximate volume 4 cc. with two capillary tubes of measured bore attached to it. This was calibrated by filling it with mercury at the temperature employed. After cleaning it was placed in a steam oven with one limb dipping into a vessel containing the molten glyceride and filled by suction. Caps were then placed over the ends of the side tubes and the instrument immersed almost completely in boiling water. When equilibrium was attained the distances of the liquid in the limbs from two marks was measured thus giving the volume.

Viscosities were measured in an Ostwald's viscometer with a second bulb containing an overflow device so that a constant volume of the hot liquid could be run through. The constant was determined at 30° with a 61·44 per cent. solution of glycerin as described in the previous paper.

Surface tensions were determined by Sugden's maximum bubble pressure method (*J.C.S.*, 1924, **125**, 28), the radii of the tubes used being approximately 0·018 and 0·335 c.m. The apparatus was calibrated at 30° with benzene and the value obtained used for the measurements at the higher temperature.

Table I summarises the values obtained for the physical properties.

TABLE I.

Physical properties of α-monoglycerides.

Glyceride	M. P.	$n^{85\cdot7}_{D}$	$d^{97\cdot3}$	$\eta^{97\cdot3}$	$\gamma^{97\cdot3}$	P.	P. calc.	Diff.
Caprylin ...	40°	1·4309	0 9646	0·0603	26·69	514	529	—15
Caprin ...	54°	1·4331	0·9399	0·0772	25·43	588	607	—19
Laurin ...	63°	1·4350	0·9248	0·0985	25·28	664	685	—21
Myristin ...	70°	1·4366	0·9121	0·1210	24·88	740	763	—23
Palmitin ...	76°	1·4484	0·9014	0·1472	25·54	823	841	—18
Stearin ...	82°	1·4400	0·8959	0·1691	25·07	894	919	—25

Temperature coefficient of refractive index 0·00038.

The variation with the number of carbon atoms is regular as may be seen from Fig. I except in the case of the surface tension which appears to fall to a minimum for myristin. The parachor P $(M\gamma^{\frac{1}{4}}/d)$ has been calculated and the value compared with those computed from Sugden's data as in the case of the triglycerides, the value 20·0 being assumed for both the non-ester oxygen atoms. The differences show a regular increase except for palmitin, and it is possible that the specimen was not sufficiently pure in spite of the special attention bestowed upon it in view of the variation of the melting point from Fischer's value.

It is noteworthy that the values found for the parachor are all lower than those calculated, whereas with the triglycerides the reverse is the case. The difference for C_2H_4 is 76, compared with 81·0 for the triglycerides and Sugden's value of 78. If the values for the monoglycerides are all raised by 2·8 per cent., the agreement between the theoretical and measured values is almost exact, but such a change would require a constant error of 11·2 per cent. in the surface tension determinations which can hardly be regarded as probable. It is possible that when dealing with these large molecules certain factors enter which are not apparent with substances of smaller molecular weight.

DOUBLE MELTING POINTS AND SOLIDIFYING POINTS.

It is well known that triglycerides exhibit double melting points, but little attention has been drawn to the corresponding phenomenon in the case of monoglycerides. Fischer (*Ber.* 1920, **53**, 1591) remarked that α-monostearin which melted at 81–82°, melted after solidification at 75–76° and similarly α-monopalmitin exhibited two melting points 78–79° and 72–73°. Brash, however (*loc. cit.*), stated that he was unable to obtain double melting points with his preparation of monopalmitin, the reason probably being that it was impure.

We have succeeded in obtaining two well-defined melting points for each of the four higher monoglycerides examined and they thus resemble the triglycerides. Measurements were made by means of the capillary tube method, a small quantity of the substance being melted in each of several thin-walled, narrow capillaries, retained above the melting point for five minutes and chilled in a freezing mixture. The tubes were then plunged into baths differing slightly in temperature in the neighbourhood of the expected melting point and the temperature of the bath which caused melting to take place within three seconds taken as the melting point of the β-form. The results appeared to be correct to within a degree.

The stability of the β-form varied greatly for different glycerides and showed a regular gradation. Thus, in the case of monostearin, the melting point was 74° and on warming to 75°, no solid appeared in 45 minutes. With monopalmitin the lower melting point was 65° and at 68° a trace of solid formed which increased very slowly. After half-an-hour at the same temperature, the contents of the tube were mainly solid. Monomyristin melts at 57° and resolidifies at a temperature just above this in about 2 minutes. On keeping the chilled melt at room temperature, a slow transformation takes place, as the melting point is found to rise with time, and on rubbing or grinding the change into the α-form is rapid. In the case of monolaurin β, melting and resolidification are almost simultaneous while with monocaprin no double melting point could be detected, doubtless owing to the extreme rapidity of the change from one form to the other.

Table II gives the melting points of the glycerides, their solidifying points (see next section) and the melting points of the fatty acids with 4 more carbon atoms than the acids from which the glycerides are derived. The corresponding values are nearly the same, a fact which is worthy of note although possibly due merely to coincidence.

TABLE II.

Melting points of α-monoglycerides and fatty acids.

Glyceride	M. P. α	M. P. β.	S. P.	Acid	M.P.
Caprylin	40	...	...	Lauric	43·5
Caprin	54	...	...	Myristic	53·6
Laurin	63	45	60·0	Palmitic	62·5
Myristin	70	57	67·0	Stearic	69·5
Palmitin	76	65	70·2	Arachidic (*n*)	75·5
Stearin	82	74	..	Behenic (*n*)	82·0

The value found for palmitin β is 8° lower than Fischer's figure while with stearin the difference is only 2°. The discrepancy in the former case may be due to the different methods employed, but considering the comparative stability of palmitin β, it appears unduly large. It has already been pointed out that the parachor for our sample is high, but at the same time the melting point agrees with that of the material used by Adam Berry and Turner and in spite of several attempts, we have been unable to obtain a specimen with a higher melting point. It seems unlikely that the true melting point could be two degrees higher as given by Fischer. To settle the question, it is intended to make another preparation using an entirely different sample of palmitic acid as the starting material.

SOLIDIFYING POINTS.

Attention has been drawn to the fact that in the case of triglycerides (*cf.* preceding paper), preparations of one substance with the same melting point may vary several degrees in their solidifying points and only after repeated recrystallization is it possible to produce a specimen with a solidifying point approximating to the melting point. Even then the two do not coincide. The same phenomenon is met with in a more marked degree with the α-monoglycerides and the present experiments point to the conclusion that the solidifying points observed are those of an equilibrium mixture of the two forms, the exact proportion depending upon very small traces of unidentifiable impurity.

The determination of the solidifying point offers considerable difficulty owing to the presence of two forms of the substance and the small amount of heat evolved on solidification. In order to obtain consistent values, it was necessary to follow the procedure adopted in the case of the triglycerides, of conducting the determination in a water-bath the temperature of which could be closely controlled so as to be always cooler than the melt but never more than 1° less. This was effected by using a comparatively small beaker of water (500 cc.) mechanically stirred and electrically heated, with hand regulation. A supplementary gas burner was used for increasing the temperature rapidly during solidification. Not less than 3 g. of the substance was filtered hot into a 3 × ½ inch hard glass test-tube fitted with a very small thermometer and placed in an air jacket in the bath. Scarcity of material precluded the use of larger quantities which would have been desirable, but the results, as well as unpublished results with triglycerides, indicate that the amount taken was sufficient to yield accurate values.

A sample of carefully purified α-monomyristin melting at 70° was first examined because the transition from one form to the other takes place fairly rapidly. A definite maximum at 62·4° was obtained in a number of experiments even when the bath was at room temperature (30°). On raising the bath temperature as previously described, the maximum rose to 63·4°, as shown in Fig. II, curve VIII. The cross on the curve marks the point at which the liquid was seeded with a crystal of the α-form. If this was not done, excessive supercooling was apt to result or a delay to occur in the transition.

On recrystallizing the specimen from ether, the melting point remained unaltered, but the maximum temperature reached rose to 65·2°. After allowing the substance to stand during 24 hours and repeating the experiment, an identical value was obtained as shown in curve X. After another recrystallization the value rose to 67·0° (curves XI & XII, the slight difference in position being due to different seeding temperatures). Further crystallization produced no appreciable change (curve XIV).

An inspection of the curves in Fig. II suggests that the maximum temperature depends to some extent upon the temperature at which a seeding crystal was added. This was shown not to be the case both in preliminary experiments and in a test made by adding some of the original material to the specimen used for experiment XII. Seeding was performed at 58°, but no temperature rise occurred until the melt had cooled at 52·4°, and the maximum attained was only 65·2°. It appears fairly certain therefore that the maximum depends upon traces of impurity too small to affect the melting point. The same impurity also seems to retard the transition to the α-form because, in general, the less pure samples had to be cooled to a lower temperature before

Fig. I

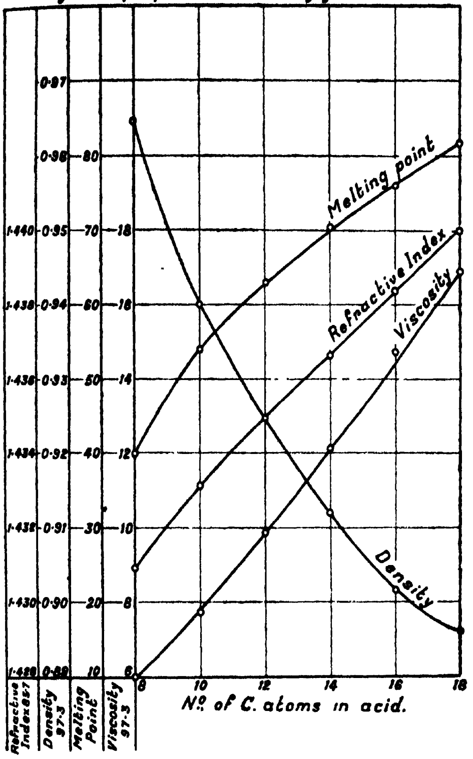

Physical properties of monoglycerides

Fig. II

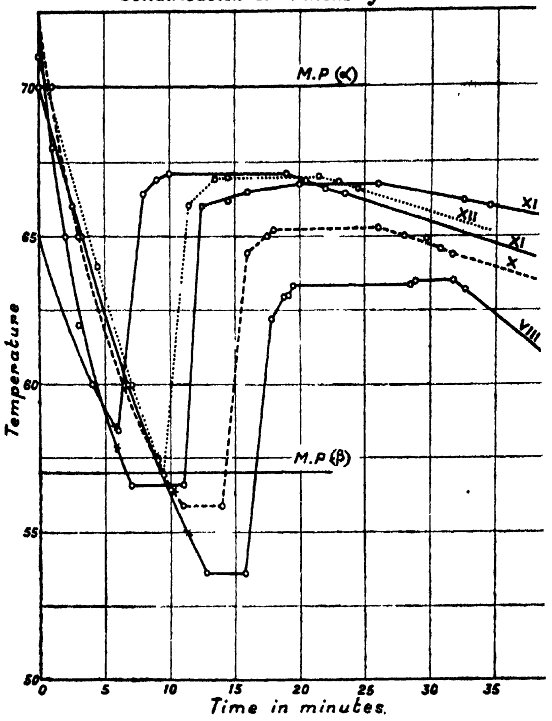

Solidification of α-monomyristin

Fig. III

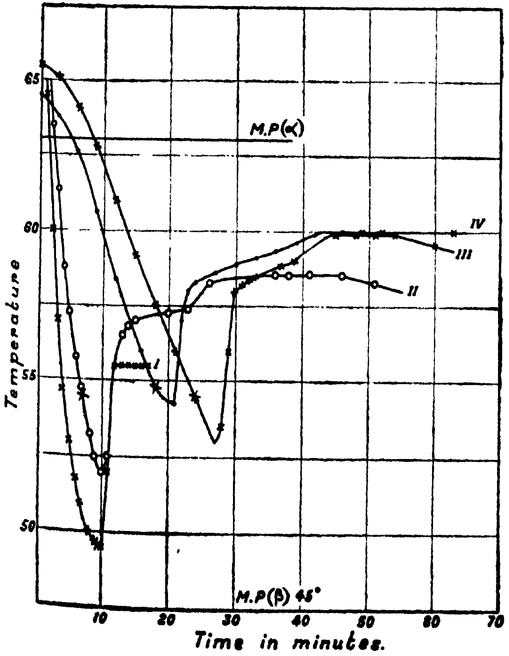
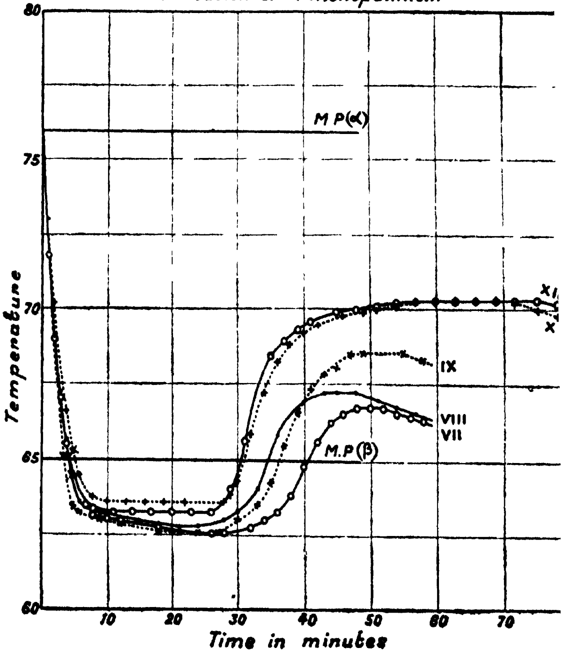

Solidification of α-monolaurin

Fig. IV


Solidification of α-monopalmitin

liberation of heat occurred. A specimen of the solid after the conclusion of experiment XIV was heated in a capillary tube and found to melt at 67·0°, the maximum temperature reached during solidification. It is thus evident that it must be an equilibrium mixture of the two forms, since the α-form melts at 70°.

Similar phenomena were observed with α-monopalmitin but owing to the slow rate of transition very careful regulation of the jacket temperature was required. For example, the first sample tested, melting at 76°, rose in temperature from 60·9 to 64·8° when the temperature of the bath was kept within 1° of that of the melt, but with the bath maintained at 55° no rise at all could be recorded. Crystallization from benzene raised the maximum temperature to 66·7 (Fig. IV curve VII) but it is doubtful if this is a true solidifying point as the maximum temperature was not maintained for any length of time. The same applies in a less degree to curve VIII obtained after a crystallization from ether. In experiment IX with the same substance the bath was maintained still nearer to the temperature of the melt and a higher maximum was reached. Three more recrystallizations from ether resulted in a steady maximum 70·3° (X), this value then remained unchanged on further crystallization (XII). The solid from this experiment melted at 70·2°.

With α-monolaurin the rise in temperature was much more sudden as might be expected from the rapidity with which the β-form changes to the α-form. After the initial rise, however, a period of slow increase in temperature was observed as may be seen in Fig. III. This suggests that there may be a third form of α-monolaurin just as there appears from the work of Loskit (*Z. physical Chem.*, 1928, **134**, 135), to be a third form of some of the triglycerides. No definite opinion can be expressed, however, without further experimental data.

The first sample rose to a maximum temperature of 55·5°; successive crystallizations from ether raised this to 58·6°, 59.9°, 60·0° and 60·0° as shown by curves I, II, III and IV in Fig. III. The curve for the last experiment was practically identical with IV and has not been drawn.

On comparing the solidifying points and melting points shown in Table II, it will be noted that the depression from the melting point of the α-form is 3° for laurin and myristin and 6° for palmitin. It has been found that the initial depression in the melting point of laurin mixed with a small quantity of palmitin or *vice versa* is 0·9° for 1 per cent. impurity in both cases, so that if the assumptions are made that the solidifying point is that of an equilibrium mixture of the α and β-forms and that the depression produced by the presence of β-form is of the same order as by the addition of a second monoglyceride (an admitted approximation), the quantity of β-form in the mixture would be about 3 per cent for laurin and myristin and 6 per cent. for palmitin.

This involves the assumption that what have been termed the α and β-forms are single bodies. According to Smidts (*Allotropy*) it is more probable that both are equilibrium mixtures. The formation of a definite equilibrium mixture with a melting point lower than that of the α-form makes it appear unlikely that the α-form itself is another equilibrium mixture, but the composition of the β-form cannot be decided without further investigation.

Another noteworthy feature is the very considerable super-cooling which appears to be necessary before the change β→α takes place even in presence of the α-form. This is probably a spurious effect due to the slow rate of transformation and the tendency of the β-form to remain in the metastable state and has been already discussed in the case of tristearin (p. 121).

In conclusion we wish to express our best thanks to Dr. J. J. Sudborough and Dr. J. L. Simonsen for valuable assistance during the course of this work.

SUMMARY.

The α-monoglycerides of the fatty acids with an even number of carbon atoms from caprylic to stearic have been made in a state of high purity and their densities, refractive indices, viscosities and surface tensions measured.

The glycerides of the four higher acids exhibit two definite melting points corresponding with those of two forms α and β. The rate of the change β→α increases with decrease in molecular weight and is almost instantaneous in the case of α-monolaurin.

The solidification point of the fused glyceride always lies below the melting point of the α-form indicating that the transformation β→α is not complete.

[Accepted, 19–6–30.]

Department of General Chemistry,
Indian Institute of Science,
Bangalore.

III.—THE SOLIDIFYING POINTS OF BINARY MIXTURES OF FATTY ACIDS AND ESTERS.

By L. A. Bhatt and H. E. Watson (with Z. H. Patel).

A knowledge of the solidifying point of binary mixtures of fatty acids or of their esters is of value for determining the proportion of the constituents in mixtures such as those obtained while fractionating a mixture of esters during the investigation of an oil. (*Cf.* Patel, Sudborough & Watson, *J. Indian Inst. Sci.*, 1923, **6**, 120.) The solidifying point of a mixture being much more sharply defined than the melting point, gives more accurate results than those which can be obtained by determining the latter property, but apart from measurements made in this laboratory very few data appear to exist except the figures for palmitic and stearic acid of de Visser (*Rec. trav. chim.*, 1898, **17**, 182).

In addition to their value for analytical purposes, solidifying point curves should give information regarding the state of molecular aggregation of the constituent substances, a problem of some complexity where the fatty acids are concerned. We have consequently made determinations for several mixtures and have compared the results with those previously obtained in this laboratory and elsewhere.

EXPERIMENTAL.

PREPARATION OF MATERIALS.

Acetic acid.—The purest commercial sample obtainable was fractionally distilled four times, the middle fraction twice partially frozen and the crystals separated, great care being taken to exclude moisture during the whole of the operations. In spite of this there appears to have been slight contamination as the product solidified at 16·4°, 16·7° being the accepted value.

n-*Butyric acid.*—A pure specimen was fractionally distilled 3 times yielding a product boiling sharply at 163° and solidifying at -8·1°.

Capric acid.—A synthetic specimen was fractionated and the middle fraction boiling at 136° at 5 mm. recrystallized 12 times from absolute alcohol. The product melted at 31·4° the accepted value, but the solidifying point was only 30·9°.

Methyl laurate and methyl myristate were prepared from fairly pure samples which had been made by fractionation of the methylated fatty acids from hydrogenated *Salvadora oleoides* (*cf.* Patel, Iyer, Sudborough and Watson, *J. Indian Inst. Sci.*, 1926, **9**A, 117). The solidifying points of these preparations were 2·5° and 14·5°. Attempts at purification by freezing were not successful and consequently the esters were fractionated at 7 mm. pressure through a four-bulbed column filled with Lessing rings. After four distillations the methyl laurate boiled within 1° and its solidifying point remained unchanged at 4·4° on refractionation. The methyl myristate required only two distillations to reach a steady solidifying point 15·9°.

Lauric and myristic acids were prepared from the purified esters by saponification with alcoholic potash and recrystallized twice from alcohol. Their solidifying points were 43·5° and 53·0°, respectively. In spite of the apparent purity of its ester, the solidifying point of the myristic acid was low, 53·8° being a better value. (*cf.* Joglekar and Watson, *J. Indian. Inst. Sci.*, 1930, **13**A. 119.)

Stearic acid.—A pure sample was recrystallized five times from 95 per cent. alcohol and the last traces of solvent removed by heating to 50° in a vacuum for some hours. The solidifying point was 69·2°.

Lignoceric acid from *Adenanthera pavonina* was twice ground with acetone and the liquid separated by suction in order to remove lower acids. The solid portion was then crystallized twice from toluene and three times from carbon disulphide. The product melted sharply at 80·8° but the solidifying point was only 79·3°. It may be mentioned that this acid is not *n*-lignoceric acid which melts at 80·5°, but appears to have a side chain, the position of which is under investigation.

DETERMINATION OF SOLIDIFYING POINTS

Solidifying points were determined by the usual method of observing maximum temperature reached by the molten substance on solidification. Temperatures were measured with thermometers divided to 0·2° or 0·1° and calibrated against standards.

For temperature control, a bath of calcium chloride solution was used above 10° and ice or a freezing mixture of ice and salt down to −15°. Between −15° and −25° calcium chloride hexahydrate and ice were found suitable and for still lower temperatures the vessel to be cooled was suspended in a tall vacuum vessel with a small quantity of liquid air at the bottom. In all cases the test tube containing the substance was surrounded by a wider one to form an air jacket. In the case of mixtures liquid at room temperature, stirring was effected with a magnetic stirrer to avoid condensation of moisture. Numerous experiments were made with different quantities of material, different bath temperatures and different degrees of supercooling. It was found that results reproducible to 0·1° could be obtained with 3 grams of material provided that the bath temperature was maintained not more than 2 or 3 degrees below that of the melt and supercooling was not excessive (about 2 degrees). All mixtures were made by adding successive weighed quantities of one constituent to the other; those containing approximately 50 per cent. of each constituent were thus arrived at in two ways, by adding A to B, or B to A, and a considerable overlap was usually allowed between the two series.

The following tables give values for the solidifying points of different mixtures, and Figs. I and II show the shape of the curves obtained on plotting these points against the percentage compositions of the mixtures. The points marked show composition by weight and the second curves drawn in several cases refer to molecular proportions. In both tables and curves, a small correction has been applied where necessary to bring the solidifying points of the pure acids to their accepted values :—

TABLE I.

Solidifying points of binary mixtures of acids and esters.

Percentage weight of first-named compound	Behenic —stearic	Lauric —stearic	Capric —stearic	Lauric —lignoceric	Lauric —myristic	Butyric —acetic	Me-laurate —me-myristate
100	79·3	43·5	31·0	43·5	43·5	— 8·1	4·4
90	76·9	38·6	26·6	43·8	39·3	—16·2	0·4
80	74·4	38·3	35·6	51·5	36·0	—24·5	—2·8
70	71·7	41·5	43·8	58·2	33·8	—33·0	—4·8
60	68·5	47·9	49·5	63·0	35·7	—28·6	—2·2
50	64·8	53·1	53·9	66·6	36·4	—18·2	1·0
40	63·6	57·9	57·4	69·5	39·2	— 8·2	4·1
30	62·3	61·6	60·8	72·2	43·3	– 1·6	7·2
20	63·0	64·5	63·7	74·6	47·0	5·0	10·4
10	65·2	67·0	66·6	76·9	50·6	11·0	12·5
0	69·3	69·3	69·3	79·3	53·8	16·7	15·9

De Visser's figures for palmitic and stearic acids and those for the pairs methyl palmitate-methyl stearate and methyl stearate-methyl behenate which have been published previously (*J. Indian Inst. Sci.*, 1923, **6**, 120 and 1926, **9**, 69) are not given in the table, but the corresponding curves are shown for the sake of comparison.

It will be noticed that the curves for all the acids are of a similar type. Starting with the higher melting acid the curvature is concave towards the X-axis. A break then occurs which appears to be more pronounced the closer the acids are to each other in the homologous series. Lauric and myristic acids are the only two exhibiting a definite minimum ; palmitic and stearic acids have a distinct flat portion in the curve ; the curves for stearic-lauric and stearic-behenic acids tend to become flat, while in the case of the others a fairly well-defined break alone is observable. After reaching a eutectic point the curves rise and are convex to the X-axis.

If the curves showing molecular percentage are examined it is seen that although the flat portion for the pairs stearic-behenic, stearic-palmitic and lauric-myristic acids corresponds very nearly to the equimolecular mixture, in no case do the two coincide. The nearest approach is shown by the last mentioned pair for which a maximum occurs at 53 mols. per cent. of lauric acid. Compound formation is thus in all probability best defined with these two acids. When the quantity of compound is relatively small, the flat portion appears when the molecular proportion of lower-melting acid is greater than 50 per cent. i.e., 60 per cent. of stearic when mixed with behenic acid and 57 per cent. of palmitic when mixed with stearic acid. For the lauric-stearic acid mixture the curve indicates a compound at about 80 per cent. of the former acid or 4 mols. to 1, while with capric and stearic acids, the ratio is still higher. It is not possible to decide whether compounds of this nature are really formed or whether the bend in the curve is displaced owing to the fact that compound formation is incomplete and that a ternary mixture of unknown composition is being dealt with.

The curves for the esters are of the simple eutectic type, methyl palmitate and stearate being the only pair with a definite break while the small inflection for methyl laurate and myristate may be due to experimental error.

The depressions in the solidifying points of pure acids on the addition of a second substance might be expected to yield data of some interest, but the results are so irregular that no deductions can be made without further experimental values. Table II gives Δ, the depression which would be produced by 100 g. of acid dissolved in 100 g.

Fig. I

Solidifying points of binary mixtures with stearic acid

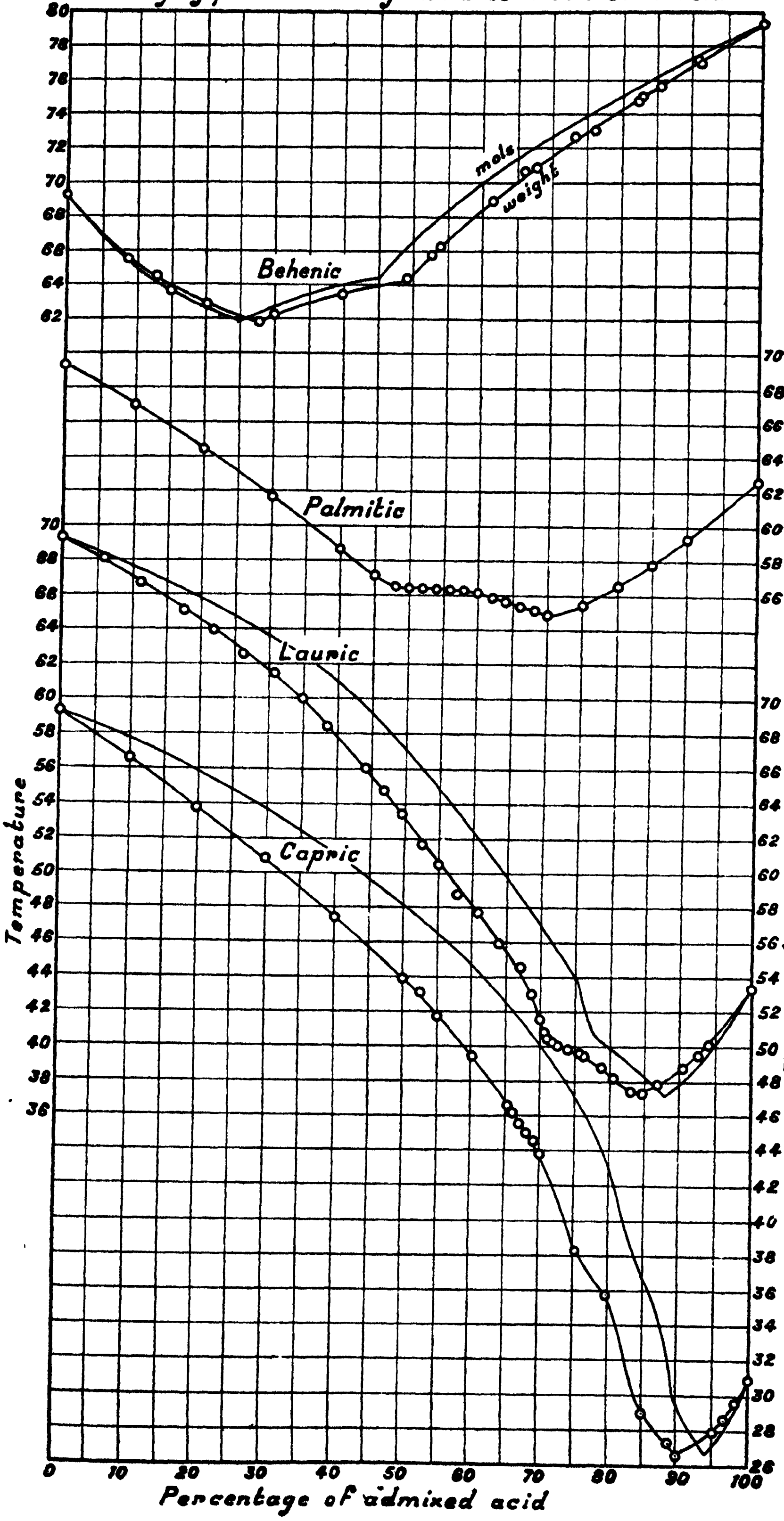
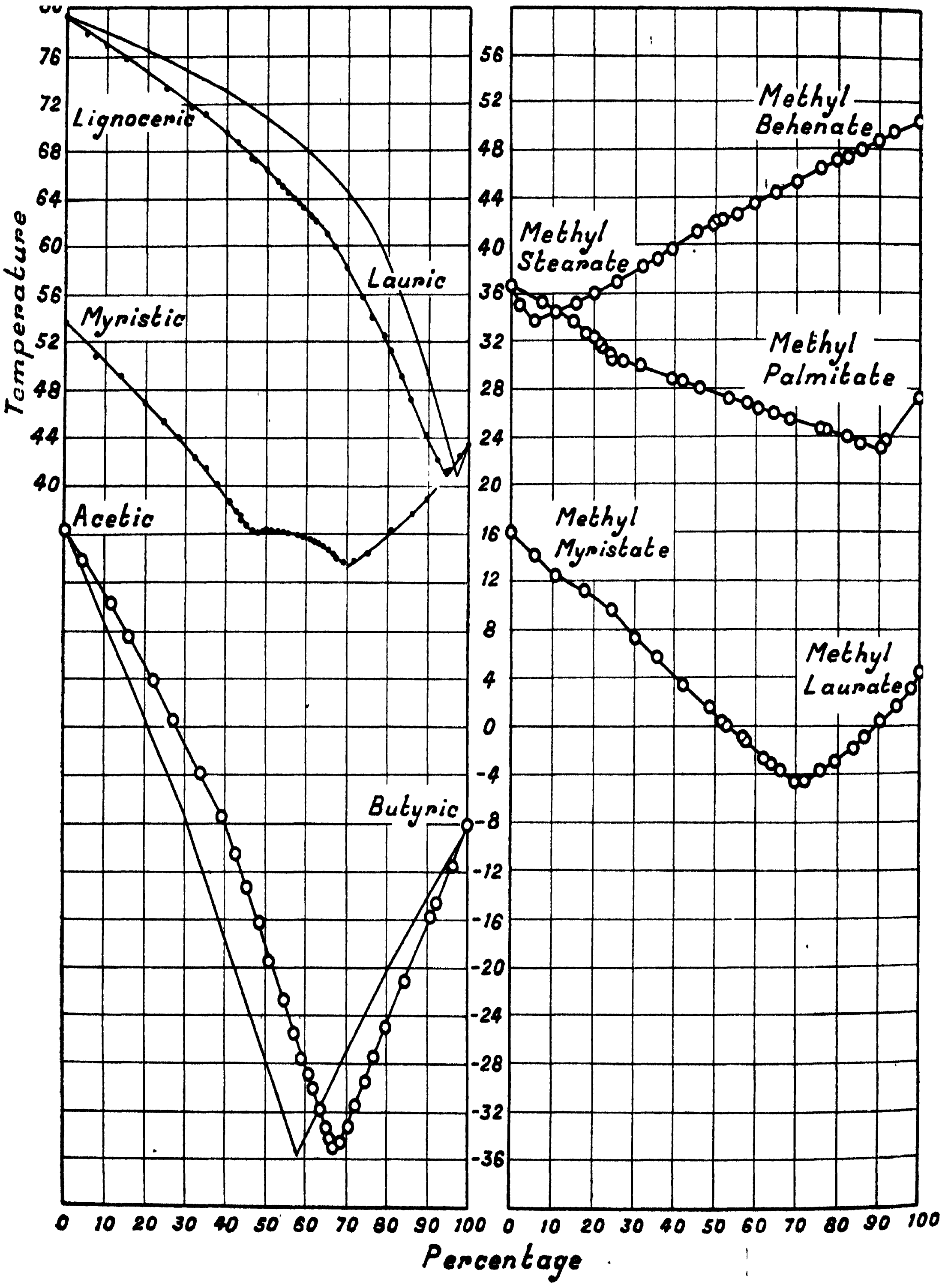


Fig. II

Solidifying point curves of acids and esters



of solvent acid if it were proportional to the depression when very small quantities were added, and *K* the depression produced by 1 gram molecule of dissolved acid with a similar assumption, *K* being thus the usual cryoscopic constant. The values are derived from the initial slopes of the curves and are necessarily very approximate.

TABLE II.

Depressions of solidifying points.

Solvent.	Solute.	Δ.	*K*.	Solvent.	Solute.	Δ.	*K*.
Stearic	Capric	24	41	Lignoceric	Lauric	23	46
Stearic	Lauric	23	46	Behenic	Stearic	24	68
Stearic	Palmitic	21	54	Palmitic	Stearic	35	100
Stearic	Behenic	45	155	Myristic	Lauric	32	64
Lauric	Myristic	40	90	Capric	Stearic	68	195
Lauric	Stearic	56	160	Butyric	Acetic	85	52
Lauric	Lignoceric	40	148	Acetic	Butyric	52	46
Me. stearate	Me. palmitate	18	48	Me. palmitate	Me. stearate	48	140
Me. stearate	Me. behenate	52	185	Me. behenate	Me. stearate	18	53
Me. myristate	Me. laurate	27	58	Me. laurate	Me. myristate	46	112

The only definite feature of these results is that for the higher acids and esters the depression produced by adding a substance of higher melting point than the solvent is very much greater than when the added substance has a lower melting point. The values of *K* for the same acid are by no means constant, but, judging from the stearic acid figures, seem to rise with rise in the molecular weight of the added acid. Δ, on the other hand, is not far from 23 when the added acid has the lower melting point and is in the neighbourhood of 40 when the converse is the case. This fact may be utilised as a very rough method of estimating the amount of impurity in an acid undergoing a process of purification but is of no value for quantitative work unless the impurity is a known acid. The cryoscopic constant has been determined by Eykman (*Z. physikal Chem.*, 1889, **3**, 203; **4**, 497) for capric, lauric, palmitic and stearic acids by dissolving naphthalene, benzophenone and anethol in them, the values being 47, 44, 44 and 45 respectively. His acids with the exception of lauric were very impure as shown by their solidifying points and consequently the results are not entirely reliable. More recently, Stratton and Partington (*Phil.*

Mag. 1924, (6) **48**, 1085), have found the value 43·13 for palmitic acid. These figures show that the values of *K* obtained when the dissolved acid has the lower melting point are approximately normal and that the high values are abnormal. The value of *K* for acetic acid is higher than the true cryoscopic constant which is 39 but no deductions can be made until further experimental values for pairs of lower acids are available. These will be determined shortly and may lead to more definite results.

Department of General Chemistry,
Indian Institute of Science,
Bangalore.

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THE SPIKE-DISEASE OF *DODONAEA VISCOSA.*

By B. N. Sastri and N. Narayana.

Dodonæa viscosa (Kanarese—*Bandarike*) is a shrub occurring wild in the *maidan* forests of Mysore. It grows to a height of about 4 ft. and possesses stipulate, lanceolate leaves with entire margin. The plant is regarded as a febrifuge in Ayurvedic medicine, but has no economic importance otherwise. It has been known to occur as a host for sandal.

This plant suffers from a disease similar to the spike of sandal (Coleman, *Dept. of Agriculture in Mysore, Myc. Bull.*, 1917, **3**, 19), prevalent in areas where sandal spike occurs. As in the case of sandal, the diseased leaves diminish in size and the internodes become shortened giving the whole plant a characteristic bushy appearance: flowering and reproduction cease and vegetative growth is accelerated.

In view of observations previously made on healthy and diseased tissues of sandal (*J. Indian Inst. Sci.*, 1928, **11A**, 23, 97 and 241), it was considered that a similar study of *Dodonæa viscosa* might throw light on the nature of sandal spike. The investigation was expected to be less complicated than in the case of sandal because the plant is not a parasite and being a shrub, presents no difficulties in the collection of samples for study.

EXPERIMENTAL.

Representative specimens of healthy and diseased plants were collected from different areas where sandal occurs, and were separated into leaves, stems and roots after thorough washing. The various tissues were dried quickly over calcium chloride in vacuum desiccators at about 0° to prevent changes due to enzyme action (Link, *J. Amer. Chem. Soc.*, 1925, **47**, 470). The dry tissues were powdered to pass a 40-mesh sieve and preserved in glass-stoppered bottles.

Methods of analysis.—Total nitrogen was determined by Gunning's modification of Kjeldahl's method to include the nitrogen of nitrates, proteins by Stutzer's copper hydroxide method and free and saline ammonia by Graffe's method (*Z. Physiol. Chem.*, 1906, **48**, 300). For the last determination, 20 g. lots of the material were treated with 100 c.c. of ammonia-free water, 100 c.c. of 95 per cent. alcohol, and 25 c.c. of saturated sodium chloride solution. Saturated sodium carbonate solution (25 c.c.) was added to the mixture which was then distilled *in vacuo* at 37° for 4 hours, ammonia being determined in the usual way.

Nitrate was estimated by Scale's method (*J. Biol. Chem.*, 1916, **27**, 327). The material (40 g.) was extracted with 80 per cent. alcohol for 36 hours and the extract after removal of alcohol, clarified with lead acetate and made to a definite volume. The nitrate in aliquot parts was reduced by a zinc-copper couple and the ammonia estimated. The Griess-Ilosway method (qualitatively) was used for nitrites.

Distribution of non-protein nitrogen.—Preliminary experiment showed that the material contained large quantities of mucilaginous matter hindering filtration of the hot-water extracts; 60 per cent. alcohol was therefore used. Weighed quantities were extracted repeatedly with boiling alcohol and the combined extracts concentrated by distillation under reduced pressure at a temperature not exceeding 40°. The concentrate was taken up with water, filtered and freed from fat by extraction with ether; it was then treated with dialysed iron, centrifuged, and the clear extract made up to a known volume.

The extract contained polypeptides, amino-acids, amides, ammonia and other forms of non-protein nitrogen. A definite volume was hydrolysed for 12 hours with sufficient hydrochloric acid to bring the concentration to 20 per cent. The insoluble melanin was separated and its nitrogen estimated. The filtrate, after distilling the hydrochloric acid was diluted, and the distribution of nitrogen determined by Plimmer's modification of the Van Slyke method (*Biochem. J.*, 1925, **19**, 1004).

Carbohydrates.—Alcoholic extracts of the material were clarified with basic lead acetate and used for the estimation of sugars. Sucrose was determined after hydrolysis with 10 per cent. citric acid, reducing sugars by Bertrand's method and starch in the residue after alcoholic extraction by hydrolysis with taka-diastase according to Davis and Daish (*J. Agric. Sci.*, 1914, **6**, 152).

Ash constituents were estimated by the A.O.A.C. methods (1925). The results of analysis are shown in Tables I–VII, the specimens having been collected from the Laggare forest area on 14th August, 1928, unless otherwise stated.

TABLE I.

Percentage of Dry Weight

	Leaves		Stems		Roots	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Total nitrogen ...	1·86	1·86	0·38	0·51	0·37	0·17
Protein nitrogen ...	1·76	1·58	0·36	0·48	0·32	0·14
Non-protein nitrogen (by difference) ...	0·10	0·28	0·02	0·03	0·05	0·03
Ash ...	5·75	6·34	3·91	3·80	6·15	2·49

TABLE II.

Percentage of total Nitrogen

Form of Nitrogen	Leaves		Stems		Roots	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Nitrate ...	0·16	0·38	2·37	4·38	1·40	2·00
Free ammonia ...	0·40	0·45	1·29	1·58	0·50	2·64
Nitrite ...	Nil.	Traces	Nil.	Nil.	Nil.	Traces

TABLE III.

Distribution of Non-protein Nitrogen.

Percentage of total Non-protein Nitrogen

Form of Nitrogen		Leaves		Stems		Roots	
		Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Melanin	Insoluble ...	10.02	11·12	8·14	11·57	9·37	14·45
	Soluble ...	5·56	4·25	4·07	4·63	4·48	1·53
Amide ...		15·50	20·54	17·66	19·50	13·75	15·16
Basic ...		25·41	19·33	24·53	19·17	15·00	14·85
Non-basic ...		47·50	47·59	44·75	43·87	55·90	55·45

TABLE IV.

Carbohydrates.

Percentage of Dry Weight

	LEAVES		STEMS		ROOTS	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Free reducing sugars as— Glucose ...	0·78	2·34	0·24	0·49	0·41	0·27
Sucrose ...	0·50	0·60	0·48	0·26	0·23	0·17
Starch ...	2·49	4·61	3·76	3·81	2·71	2·86

TABLE V.

Ash Constituents.

Percentage of Dry Weight

Date of collection	Locality	Tissue—condition	Ash	Total nitrogen	P_2O_5	Ca	K_2O
14—11—27	Laggare ...	Leaf, H	6·20	1·85	0·10	0·38	0·47
,,	,, ...	,, D.	9·65	1·76	0·15	0·15	0·55
15—4—28	Denkanikota ...	,, H.	4·96	1·95	0·11	0·77	0·45
,,	,, ...	,, D.	6·27	2·29	0·15	0·10	0·46
15—7—28	,, ...	,, H.	6·09	2·36	0·52	0·95	0·32
,,	,, ...	,, D.	6·43	1·91	0·53	0·77	0·48
23—8—28	Uttarahalli ...	,, H.	6·57	2·41	0·37	0·59	...
,,	,, ...	,, D.	5·17	1·82	0·39	0·56	...
14—9—28	Laggare ...	,, H.	5·75	1·86	0·52	0·56	0·14
,,	,, ...	,, D.	6·34	1·85	0·32	0·37	0·12
,,	,, ...	Stem H.	3·91	0·38	0·39	0·32	0·04
,,	,, ...	,, D.	3·80	0.51	0·29	0·27	0·03
,,	,, ...	Root H.	6·15	0·37	0·25	0·63	0·19
,,	,, ...	,, D.	2·49	0·17	0·24	0·36	0·15

H = healthy ; D = diseased ; .... not determined.

Discussion.—It may be seen from Tables I, II and III, that the different forms of non-protein nitrogen, such as nitrate, amide and ammonia are higher in the diseased tissues than in the healthy ones. Bourquet obtained similar results in his study of the virus diseases, the mosaic of tobacco, the curly-top of sugar beet and the leaf-roll of potato (*J. Amer. Chem. Soc.*, 1916, **38**, 2572). The present observations suggest that the spike of *Dodonæa viscosa* is allied to the above and also caused by a virus.

Table IV shows that, as in the case of sandal spike (*J. Ind. Inst. Sci.*, 1928, **11A**, 99) diseased leaves have higher sugar and starch contents than the healthy ones. Similar results were obtained in another series of experiments in which tissue-fluids were expressed from the leaves, as may be seen from Table VI. The diastatic activity of the juice was measured and found to be higher for the diseased plant as in the case of sandal.

TABLE VI.

Specimens collected from the Uttarahalli forest area on 23-8-1928.

Carbohydrate	Healthy	Diseased
Free reducing sugars in 100 cc. of fluid ...	301 mg.	341 mg.
Sucrose ,, ...	114 ,,	225 ,,
Diastatic activity mg. of maltose*	6·7 ,,	24·0 ,,
Starch in residue ...	6·1 per cent.	8·4 per cent.

* Vide *J. Ind. Inst. Sci.*, 1928, **11A**, 25.

The figures for nitrogen, phosphorus and potassium (Table V) do not appear to depend upon the condition of the plant. Those for calcium are, however, more significant, being invariably lower in the case of diseased tissues. It is well known that calcium assists the hydrolysis and translocation of starch and prevents the accumulation of organic acids such as oxalic, which would otherwise prove poisonous. The growing parts of plants require large quantities of calcium, and serious metabolic disturbances are caused by a shortage of the element. The high N/Ca ratio in the diseased tissues is indicative of pathological disturbances (Parker and Truog, *Soil Sci.*, 1920, **10**, 49).

To determine whether the differences in mineral composition observed in the cases of healthy and diseased tissues of sandal and *Dodonæa viscosa* are borne out in the case of *Zyzyphus œnoplia*, known also to suffer from a similar disease, specimens of the latter were analysed for their nitrogen, phosphorus and calcium contents, percentage on the weight of dry leaves being shown in Table VII.

TABLE VII.

Analysis of Leaves of Zyzyphus œnoplia.

Date	Locality	Condition of the leaf tissue	N	P_2O_5	Ca
5—1—28	Aiyur	Healthy	2·47	1·53	0·89
,,	,,	Diseased	3·68	1·87	0·38
5—3—28	Denkanikota	Healthy	3·20	1·07	1·15
,,	,,	Diseased	3·87	1·28	0·47

Calcium is again lower in the diseased tissues, and measurements of diastatic activity showed that this factor was higher than in the healthy leaf fluid.

SUMMARY.

The non-protein nitrogen constituents of the tissues of *Dodonæa viscosa* in healthy and diseased conditions were determined. The nitrate, nitrite, ammonia and amide contents of the diseased tissues were invariably higher than those of the corresponding healthy ones.

As in the case of sandal, sugars and starch tend to accumulate in the diseased tissues. The diastatic activity of the latter was also higher than that of the healthy tissues.

The diseased condition is further characterised by deficiency in calcium. This is true also of *Zyzyphus œnoplia* which suffers from a similar disease.

In conclusion, our thanks are due to Prof. Roland V. Norris, for his keen interest in the work and to Mr. M. Sreenivasaya for many helpful suggestions.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS.

PART III. The Globulins of Bengal Gram (*Cicer Arietinum,* Linn.) and Horse Gram (*Dolichos Biflorus*).

By Nuggihalli Narayana.

Bengal gram is an important Indian foodstuff belonging to the order *Leguminosæ*. The vernacular names are :—Sanskrit, *Chenaka*; Hindi, *Chana*; Tamil, *Kadalay*; and Telugu, *Senagalu*. The crop is raised in all parts of India, the upper basins of the Ganges and the Indus being the chief gram-producing areas. Agricultural statistics show that this pulse accounts for about 13 per cent. of the total area (including States) under food crops. It is consumed by all classes of people and is believed to be highly nutritious. In many parts of the country any surplus material is fed to cattle.

Horse gram is one of the cheapest among Indian pulses and is chiefly employed as food for horses and cattle. The vernacular names are :—Sanskrit, *Kulattha*; Hindi, *Kulthi*; Kanarese, *Hurali*; and Tamil, *Kollu*. It is grown in all parts of the country as a catch-crop and thrives with a minimum rainfall. As it enriches the soil with nitrogen, the plant, after harvesting the seed, is generally ploughed in as green manure.

The present investigation of the composition of the globulins which form the chief proteins of the seeds, was undertaken in view of the importance of the pulses in relation to nutrition.

EXPERIMENTAL.

The seeds were sun-dried until crisp and easy to handle. The husk was removed from the Bengal gram and the kernel ground to obtain the flour. The horse gram was ground whole because (*a*) it was not possible to remove the husk and (*b*) the whole seed is invariably fed to animals. Flours passing the 60-mesh sieve were used in the present investigation, specimens dried at 100° giving the following on analysis, (Table I) :—

TABLE I.

Pulse	Ash	Ether extractives	Crude fibre	Crude protein (N x 6·25)	Carbohydrates (by difference)	True protein (determined separately)
Bengal gram ...	2·45	4·72	1·13	28·14	63·56	25·04
Horse gram ...	3·51	2·32	5·48	26·40	62·29	21·93

Extraction of the Globulins.—Preliminary trials with Bengal gram showed that the optimum concentration of salt solution was 6 per cent. which extracted, in 4 hours at 25–30°, about 88 per cent. of the total nitrogen. In the case of the horse gram, the most suitable concentration was 8 per cent. which extracted nearly 80 per cent. of the total nitrogen in 2 hours.

PREPARATION OF THE GLOBULINS.

Extraction.—In each case about one kilogram of the flour was treated with 6–8 litres of the required concentration of sodium chloride solution and stirred vigorously for 2–3 hours. The mixture was then strained through cheese cloth, the liquid poured into large fluted gravity filters and allowed to stand overnight, toluene being added to prevent bacterial action. About 2–3 litres of slightly coloured, opalescent extract was thus obtained.

Precipitation.—The globulins were precipitated from the extract by (1) dialysis, (2) acidification and (3) salting out. The extract was dialysed against cold running distilled water for 4–6 days until the dialysates were free from traces of chlorides. The globulin of the Bengal gram then precipitated readily but that from horse gram did so only on adding a small quantity of acetic acid. The proteins were separated by centrifuging, redissolved in salt solution and dialysed as before. (2) The extract was diluted until cloudy, saturated with carbon dioxide and treated with a few drops of acetic acid to complete the precipitation of protein. (3) This method was adopted only in the case of Bengal gram, the extract being saturated with ammonium sulphate. The precipitated protein was filtered, and washed repeatedly with saturated ammonium sulphate solution. It was then peptised by shaking with sufficient water and dialysed against distilled water until the dialysate was free from sulphate.

Purification.—Suspensions obtained by the different methods were centrifuged and washed repeatedly by triturating with distilled water; they were then dehydrated by washing with graded strength of alcohol, and finally with ether, the dry preparations being powdered and passed through the 100-mesh sieve preparatory to further examination.

GENERAL PROPERTIES.

The preparations from horse gram were light brown, and those from Bengal gram cream-coloured. All gave reactions characteristic of proteins, and contained sulphur, tyrosine and tryptophan, but no phosphorus. They were completely soluble in dilute alkali and glacial acetic acid. Elementary analysis of the preparations gave the following percentages (Table II), sulphur being determined by the method of Hoffman and Gortner (*J. Amer. Chem. Soc.*, 1923, **45**, 1033).

TABLE II.

	From Bengal gram by			From Horse gram by	
	(1)	(2)	(3)	(1)	(2)
Combined moisture ...	8·73	8·05	7·65	11·50	12·36
Ash	0·33	0·33	0·34	0·48	0·64
Nitrogen *	17·05	17·26	17·17	15·75	15·90
Sulphur *	0·36	0·35	0·36	0·53	0·59

* On ash and moisture free basis.

Nitrogen distribution was determined by the method of Van Slyke as modified by Plimmer and Rosedale (*Biochem. J.*, 1925, **19**, 1004). Arginine in the diamino-fraction was estimated by the method of Plimmer (*Biochem. J.*, 1916, **10**, 115), and sulphur according to Plimmer and Lowndes (*Biochem. J.*, 1927, **21**, 247). Arginine was also estimated directly in the hydrolysate, Plimmer and Rosedale having observed (*Biochem. J.*, 1925, **19**, 1020) that higher values than those by the Van Slyke method are thus obtained. *Free amino-nitrogen* was estimated in 1 per cent. solutions of the globulins in dilute alkali. The Van Slyke micro-apparatus was used and 30 minutes allowed for the reaction. The results of the foregoing determinations are given in Table III.

TABLE III.

	From Bengal gram by			From Horse gram by	
	(1)	(2)	(3)	(1)	(2)
Nitrogen as :—					
Acid Insoluble melanin ...	0·32	0·32	0·48	1·32	1·30
Soluble melanin (absorbed by lime)	0·38	0·30	0·29	0·05	0·05
Amide	10·40	10·40	10·47	10·09	10·78
Basic :—					
Arginine	19·23	19·24	19·47	12·61	11·88
Histidine	1·75	1·12	1·82	1·13	1·75
Cystine	0·24	0·27	0·24	0·41	0·45
Lysine	8·45	8·80	8·22	9·56	8·93
Non-basic :—					
Amino	57·80	58·43	58·45	63·64	63·84
Non-amino	0·49	0·47	0·46	1·88	1·65
Total ...	99·06	99·35	99·90	100·69	100·59
Arginine direct	22·51	22·50	23·17	14·09	14·01
Free amino-nitrogen, direct ...	4·99	5·23	...	4·81	4·27
Half Lysine, N.	4·23	4·40	...	4·78	4·47
Sulphur in basic fraction ...	0·55	0·59	0·54	...	...
Sulphur in non-basic fraction ...	1·60	1·44	1·44	...	...

TYROSINE, TRYPTOPHAN AND CYSTINE.

Tyrosine was estimated by the method of Zuwerkalao (*Z. physiol. Chem.*, 1926, **163**, 185) and tryptophan by that of Komm (*Z. physiol. Chem.*, 1926, **163**, 202). The figures for cystine obtained by the Van Slyke method cannot be taken as correct, since, owing to racemisation. during hydrolysis, the amino-acid is incompletely precipitated by phosphotungstic acid. Independent determinations of cystine were therefore carried out by the method of Folin and Looney (*J.Biol. Chem.*, 1922, **51**, 421). Tryptophan in the globulin of horse gram could not be estimated by the method of Komm, as the colour obtained with the globulin was different in shade from that with pure tryptophan. The results were as follows :—

TABLE IV.

Percentages of ash and moisture-free protein.

	Globulin from	
	Bengal gram	Horse gram
Tyrosine	4·90	6·68
Cystine	0·88	1·81
Tryptophan	0·41	present
Arginine (Van Slyke) ...	10·27	6·02
Arginine (direct)	12·09	7·11
Histidine	0·99	0·84
Lysine	7·57	7·64

DISCUSSION OF RESULTS.

The figures for the distribution of nitrogen show that the different preparations of each globulin are identical in composition. Arginine, as estimated in the protein hydrolysate, is 2–3 per cent. higher than when estimated in the diamino-fraction, showing that the amino-acid is not completely precipitated by phosphotungstic acid (Plimmer and Rosedale, *loc. cit.*).

The ratio of sulphur in the basic and non-basic fractions of the globulin from Bengal gram is nearly 1 to 3; if all sulphur in the protein had been present as cystine, the ratio should have been 1 to 1·5 (Plimmer and Lowndes, *loc. cit.*). Moreover, since cystine estimated by the method of Folin and Looney (*loc. cit.*) accounts for only two-thirds of the total sulphur, it may be inferred that in addition to cystine, one or more sulphur-containing compounds are present in the non-basic fraction.

The close agreement obtained between the figures for free amino-nitrogen and half the lysine nitrogen in both globulins indicates that the free amino-group of lysine accounts for all the nitrogen in that form so that practically no other free amino-groups are present in the proteins. This result is a contrast with an unpublished observation of the author that in the case of the prolamin of Ragi (*Eleusine coracana*), only one-seventh of the free amino-nitrogen is due to lysine.

Both the globulins contain sufficient amounts of tyrosine, arginine and lysine, while cystine and tryptophan contents are comparatively low. A comparison with the composition of similar proteins present in other important Indian pulses (*J. Indian Inst. Sci.*, 1929, **12A**, 193; *J. Biol. Chem.*, 1920, **42**, 59) shows that the globulin of the Bengal gram is characterised by a high percentage of arginine and a low one of non-amino nitrogen (proline ?).

SUMMARY.

The globulins of Bengal and horse gram were isolated and analysed by the method of Van Slyke. Tyrosine, tryptophan and cystine were estimated. The arginine content of the Bengal gram, was much higher than those usually met with in the globulins of other Indian pulses. Both proteins were found to contain sufficient amounts of arginine, tyrosine and lysine, but were deficient in cystine and tryptophan.

Department of Bio-Chemistry,
Indian Institute of Science,
Bangalore.

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AMYLASE FROM WHEAT.

By Dattatreya Vishnu Karmarkar and Vinayak Narayan Patwardhan.

Lintner (*J. pr. Chem.*, 1890, **41**, 91) first described the amylase from ungerminated wheat; he found it similar to that from barley malt, but without liquefying power. Chrzaszcz (*Biochem. Z.*, 1923, **142**, 147) observed that the optimum temperature for the amylase from wheat malt lay between 49° and 55°. Naylor, Spencer and House (*J. Amer. Chem. Soc.*, 1925, **47**, 3037) noted that the amylase from germinated wheat was highly active and that its optimum reaction lay between P_H 4.5 and P_H 5.1. We found the amylase from germinated wheat to be about four times as active as that from barley malt: the optimum temperature zone was between 49° and 58°, and the optimum reaction, P_H 4·6.

That dry malt-amylase does not suffer loss of activity if heated to 100°, but is completely inactivated at 170° was first observed by Hueppe (*Pharm. Zentr.*, 1887, **22**, 488). We found that heating dry wheat-amylase for 1 hour at 160° was sufficient to inactivate it. Temperatures up to 110° did not much affect the activity, but with further rise in temperature, the activity diminished steadily and was completely lost at 160°.

In the course of their attempts to purify malt-amylase, Sherman and Schlessinger (*J. Amer. Chem. Soc.*, 1915, **37**, 643) observed that loss of activity during dialysis was unavoidable. Patwardhan (*J. Indian Inst. Sci.*, 1929, **12A**, 185) observed that maize-malt lost its activity on prolonged dialysis in collodion bags. Patwardhan and Narayana (*J. Indian Inst. Sci.*, 1930, **13A**, 38) found that ragi-amylase behaved in a similar manner. We found that germinated wheat-amylase also lost nearly all activity when subjected to continued dialysis in collodion bags; the activity could not be restored by addition of salts, or buffer solutions, or amino-acids.

Sherman and Walker (*J. Amer. Chem. Soc.*, 1919, **41**, 1867; 1921, **43**, 2461) found that aspartic acid, asparagine, glycine, alanine, phenylalanine and tyrosine caused marked increase in the rates of hydrolysis of starch by purified pancreatic amylase, commercial pancreatin, saliva, and purified malt-amylase. Malt extract, however, remained unaffected by the addition of amino-acids. The above authors suggested that amino-acids act as protectors against the destruction of the enzyme in aqueous solutions, but did not show how the destruction actually took place.

Wroblewski, (*Z. physiol. Chem.*, 1898, **24**, 173) showed that in weak alkaline solutions trypsin did not inactivate diastase, but that pepsin destroyed it in weak acid solutions. Biedermann (*Biochem. Z.*, 1922, **127**, 38) found that salivary amylase was inactivated by pepsin though it resisted the action of trypsin. We found that preparations of amylase from germinated and ungerminated wheat were rendered inactive by pepsin, the rate of inactivation increasing with the quantity of pepsin added. Under similar conditions, however, pepsin failed to inactivate amylases from barley, cholam, maize and ragi: trypsin and papain were also without any action.

The influence of salts on amylases has lately led to some controversy. Sherman and Thomas (*J. Amer. Chem. Soc.*, 1915, **37**, 623) found that the chlorides, sulphates, nitrates and phosphates of sodium and potassium greatly accelerates the hydrolysis of starch by amylases. Koga (*Biochem. Z.*, 1923, **142**, 159) observed no activation and used the absence of response by plant amylases to stimulation by sodium chloride as a criterion for distinguishing them from animal amylases. Eadie (*Biochem. J.*, 1926, **20**, 1016) observed that high concentrations of salts only partially restored the activity lost by malt and pancreatic amylases during dialysis. One of us (Patwardhan, *J. Indian Inst. Sci.*, 1929, **12A**, 185) found that sodium chloride was without any regenerating action on inactivated amylase from maize. Narayanamurti (*J. Indian Inst. Sci.*, 1930, **13A**, 63) found the same salt inhibitive to purified cholam-malt amylase when its concentration exceeded 0·0000166 *N*. Our experiments with salts of different inorganic and organic acids show that under no condition could any of them activate wheat-malt amylase.

EXPERIMENTAL.

Malting and Preparation of the Enzyme.—In view of the observations of Prokopenko (*Deut. landw. Rundschau*, 1928, **1**, 824) that the amylase content of wheat-malt is independent of the temperature of germination, no special precautions were adopted. The details with regard to steeping and germination of the grain, drying and storage of the malt, and precipitation and preservation of the enzyme were the same as those described in an earlier communication (Patwardhan, *loc. cit.*).

Kahlbaum's pure soluble starch was used after repeated washing with water and decantation until the reducing action was constant; it was then filtered and dried. Sugar was estimated by the method of Willstätter and Schudel (*Ber.*, 1918, **51**, 780).

Comparative activity of barley and wheat-malt extracts.—The extracts were prepared by shaking the respective malts (2 gms.) with

Fig. I

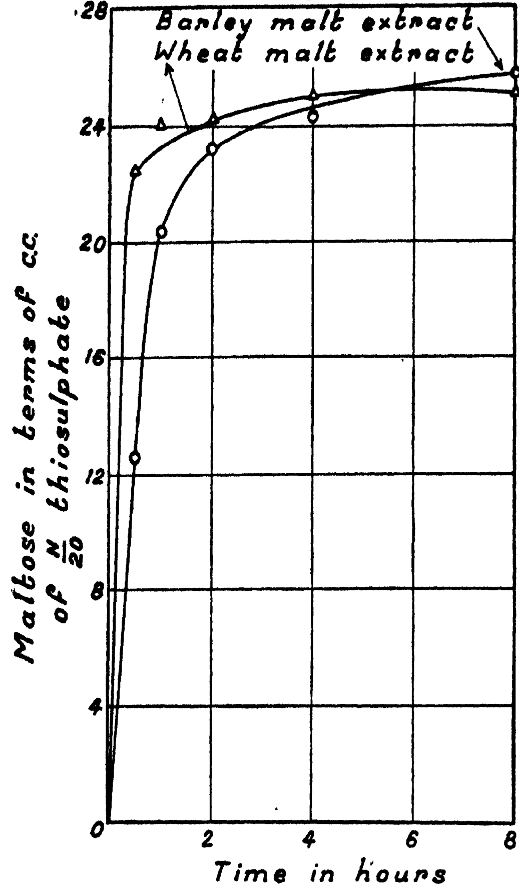


Fig. II

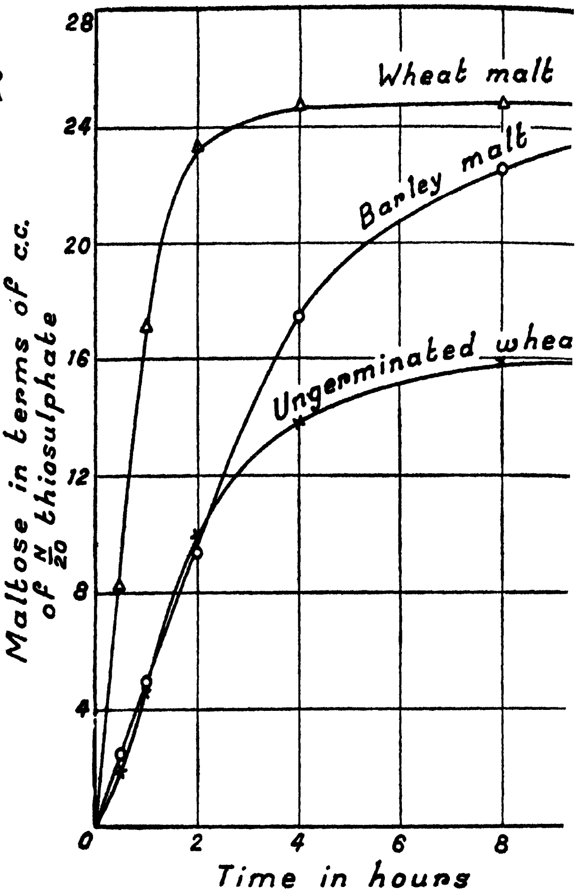


Fig. III

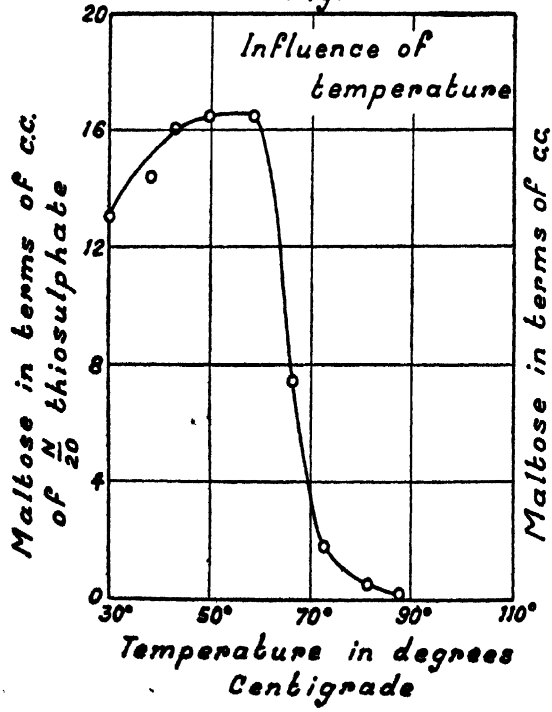
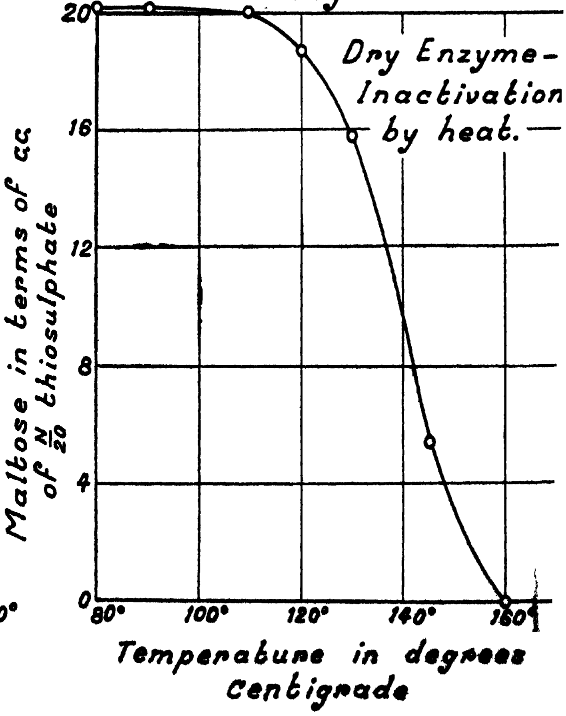


Fig. IV



water for about one hour, filtering and bulking to 100 c.c. Starch solution (2 per cent.) was distributed in quantities of 100 c.c., one group receiving 20 c.c. of wheat-malt extract each, and the other 20 c.c. of barley malt extract each; toluene was added and the mixtures incubated at 38°. At regular intervals, aliquot parts were removed and their sugar contents determined. The results have been plotted in Fig. I.

Comparative activity of amylases from barley malt, germinated wheat and ungerminated wheat.—Experimental details were as above, except that solutions (0·02 per cent.) of the precipitated enzymes were added in place of malt extracts. The results have been plotted in Fig. II, showing that germinated wheat-amylase is more active than the corresponding barley-enzyme during the first four hours; in the later stages the activities are very nearly equal.

EFFECT OF TEMPERATURE ON WHEAT-MALT AMYLASE.

Enzyme in aqueous solution.—A 2 per cent. starch solution (45 c.c.) at nine different temperatures was treated with 5 c.c. of enzyme solution (0·0375 per cent.). Sugar was estimated after hydrolysis for 30 minutes. The results plotted in Fig. III show that the optimum zone lies between 49° and 58°; above this the enzyme is steadily weakened until completely inactivated at 88°.

Dry enzyme.—Specimens of the dry enzyme in weighing bottles were kept at different temperatures for one hour. They were then cooled to the room temperature and their respective activities determined in the manner described in the last experiment with the difference that the concentration of the enzyme was 0·05 per cent. and the time of incubation 1 hour. The results (Fig. IV) show that above 110° the enzyme weakens rapidly and is finally killed at 160°.

RESPONSE TO CHANGE IN HYDROGEN-ION CONCENTRATION.

To each of twelve flasks containing 40 c.c. of starch solution (2 per cent.) and 20 c.c. McIlvaine's phosphate and citric acid buffer of different reactions, 20 c.c. of enzyme solution (0·01674 per cent.) were added and the mixtures incubated at 38° for one hour. Sugar was then determined in aliquot portions. The results (Table I) show the optimum reaction at P_H 4.6.

TABLE I.

P_H	3·8	4·0	4·2	4·4	4·6	4·8	5·0	5·2	5·6	5·8	6·0	6·9
Maltose (c.c. *N*/20 thiosulphate).	8·8	9·6	10·2	11·6	11·7	11·45	11·25	11·0	9·5	8·6	8·0	7·2

EFFECT OF CONCENTRATION.

Enzyme.—To a number of flasks containing 50 c.c. of starch solution (2 per cent.), varying quantities of the enzyme solution (0·1674 per cent.) were added, and the volume made up to 60 c.c. by distilled water; after incubation for one hour at 38°, sugar was estimated, Fig. V showing that the rate of hydrolysis increased with concentration of the enzyme up to a maximum and then remained constant.

Substrate.—Seven flasks each containing starch solution (50 c.c.) with concentration between 0·5 and 6·0 per cent. were incubated with 10 c.c. of enzyme solution (0·01 per cent.) at 38°. Sugar was estimated after one hour and Fig. VI shows that the range of optimum substrate concentration is 2–4 per cent.

INACTIVATION OF THE ENZYME DURING DIALYSIS.

Bags were prepared from 6 per cent. collodion, and into each 10 c.c. of enzyme solution (0·10 per cent.) was pipetted and dialysed against distilled water saturated with toluene, for several days at room temperature, along with control tubes containing 10 c.c. of enzyme and toluene. At each stage of observation, two collodion bags and one control tube were taken out, their respective contents made up to 50 c.c. and activities tested against 2 per cent. starch solution.

The results (Fig. VII) show that dialysis diminishes the activity to a negligible figure in a fortnight, while the undialysed enzyme remains unaffected.

INFLUENCE OF PEPSIN, TRYPSIN AND PAPAIN ON THE ACTIVITY.

To determine whether the inactivation observed on prolonged dialysis was due to hydrolysis of the enzyme protein, the following experiments were conducted.

Two specimens (50 c.c.) of 0·02 per cent. amylase were treated with 10 c.c. of trypsin solution (0·5 per cent.); to a third, 10 c.c. of boiled trypsin was added. The flasks were incubated at 38° after addition of toluene. Similar trials were also carried out with amylase solutions to which fresh and boiled solutions of pepsin and papain, respectively, had been added. After 28 hours, aliquot parts from the different experiments were transferred to nine other flasks each containing 50 c.c. of starch solution (2 per cent.) and the respective amylase activities determined after incubation for 1 hour at 38°.

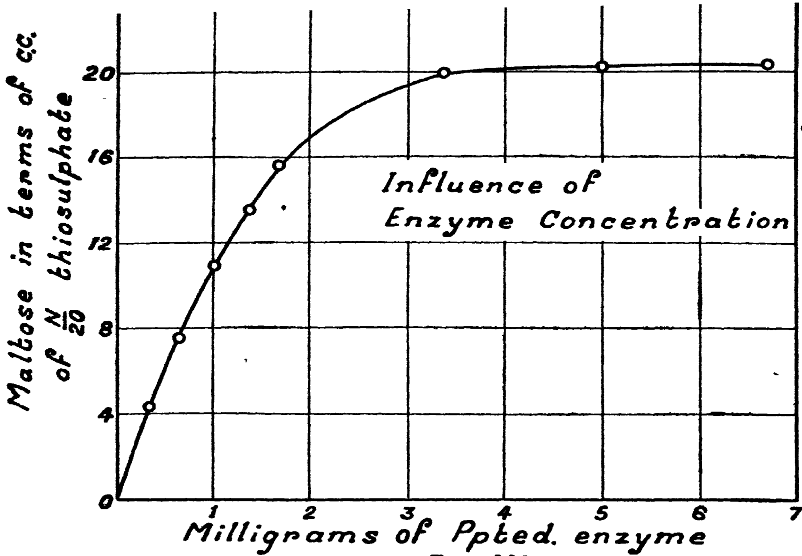


Fig. VI

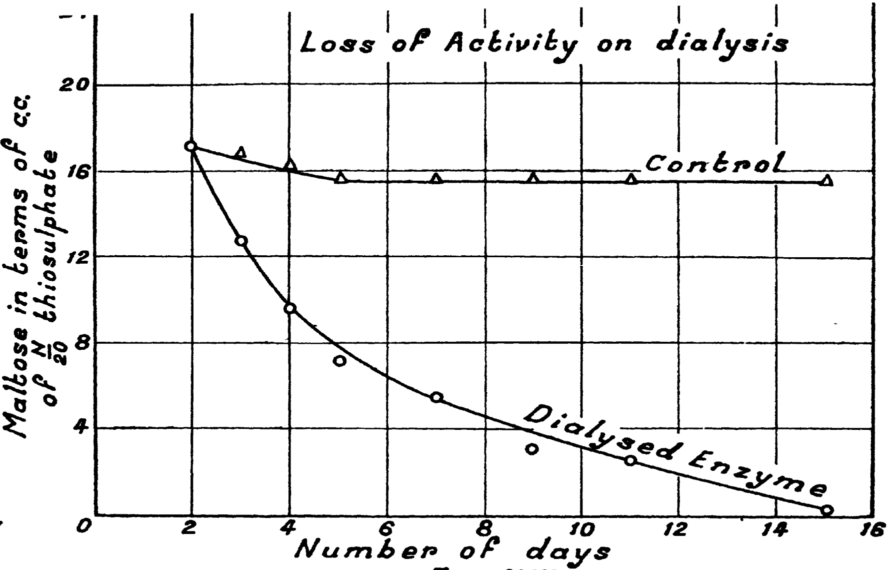
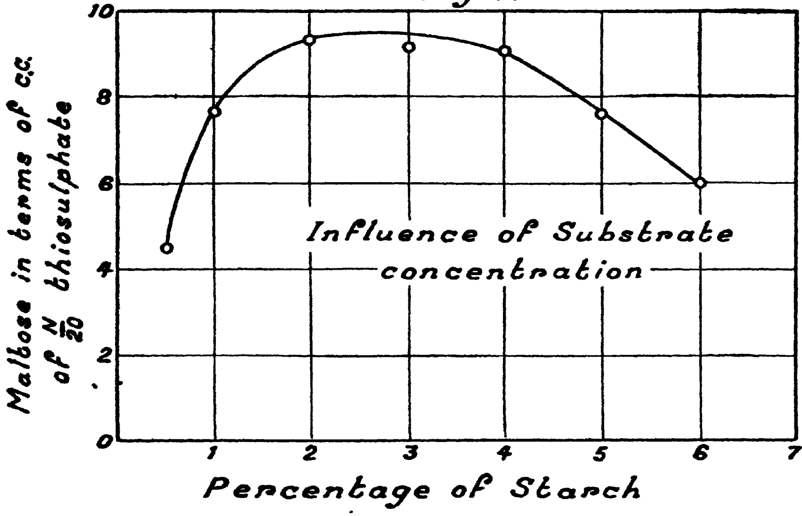
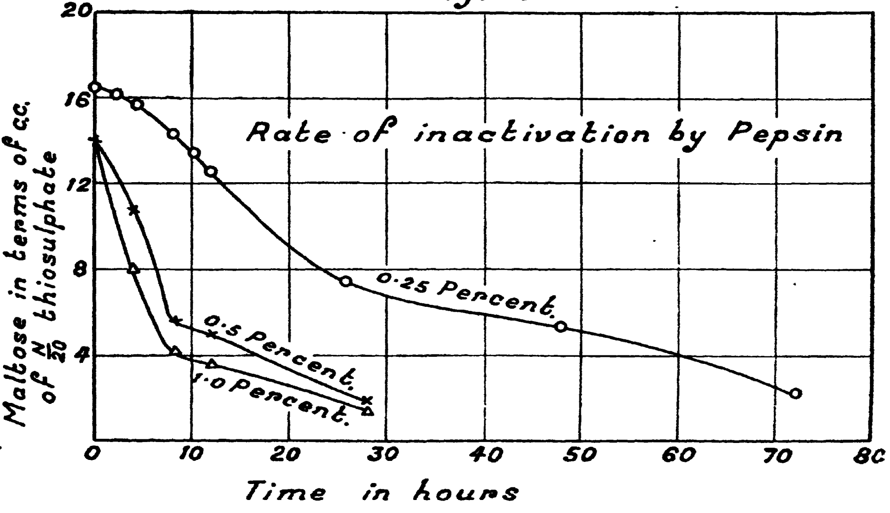


Fig. VIII





The results (Table II) show that there was marked inactivation in presence of pepsin while trypsin and papain had no effect.

TABLE II.

Proteolytic enzyme	Mgms. of maltose in terms of c.c. thiosulphate		
	Unboiled		Boiled (control)
	I	II	
Pepsin	4·1	2·1	17·9
Papain	19·0	19·2	20·7
Trypsin	17·4	17·8	20·1

Rate of inactivation by pepsin.—To two specimens (25 c.c.) of the enzyme solution (0·2 per cent.), 25 c.c. of 1·0 and 2·0 per cent. solutions respectively of pepsin was added with 1 c.c. of toluene. At stated intervals, 5 c.c. was removed and diluted to 25 c.c., 10 c.c. of this being mixed with 40 c.c. of starch solution (2 per cent.) and incubated at 38° for one hour after addition of toluene. The results (Fig. VIII) show that the inactivation was most rapid in the first 10 hours; it then slackened and was nearly complete at the end of 28 hours.

INFLUENCE OF SALTS ON ACTIVE AND INACTIVE ENZYME.

Active enzyme.—Several mixtures containing 40 c.c. of starch solution (2 per cent.), 20 c.c. of McIlvaine's buffer of P_H 4·6, 10 c.c. of the enzyme solution, 10 c.c. of a salt solution of a particular concentration and toluene, were arranged. The salts used were the chlorides of sodium, potassium and calcium and the sulphate, oxalate, malate and tartrate of potassium. They were added in such quantities as to give final concentrations of 0·0025, 0·005 and 0·01 molar. Adequate controls were also maintained. After incubation at 38° for 1 hour the sugar contents were determined.

No differences being observed, it is inferred that, under the experimental conditions, salts were without any influence on the activity of wheat-amylase.

Inactive enzyme.—The details were the same as those of the previous experiment except that amylase inactivated by prolonged dialysis was substituted for the active enzyme. In no case could any activity be observed.

INFLUENCE OF AMINO-ACIDS.

The procedure was the same as that adopted in the previous experiment. Sodium salts of glycine, alanine, aspartic acid, hippuric acid and leucine were added in such quantities as to make the final concentrations 0·0025, 0·005 and 0·01 molar. It was observed that neither the active enzyme nor the one inactivated by prolonged dialysis was appreciably influenced by the amino-acids.

SUMMARY.

1. Amylase from germinated wheat is more active than the corresponding barley enzyme.

2. The optimum temperature for the action of wheat-amylase lies between 49° and 58°. If heated for 1 hour at 160° the dry enzyme is completely inactivated. The optimum reaction is P_H 4·6.

3. The enzyme is inactivated on prolonged dialysis in collodion bags. The activity is not regenerated by adding various salts and amino-acids.

4. Neutral salts and amino-acids are without any influence on the activity of wheat-amylase. Pepsin inactivates the enzyme, but trypsin and papain do not.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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PART IV.—BIOLOGICAL OXIDATION OF SULPHUR.

Influence on Ammonification and Nitrification in Activated Sludge.

By C. V. Ramaswami Ayyar.

With the recent advances in improved systems of sewage disposal, the importance of nitrogen transformations in sewage has been recognised (*J. Indian Inst. Sci.*, 1927, **10A**, 99), but the influence of other processes such as sulphur-oxidation on the nitrogen cycle in activated sludge has not been so well understood as in the case of soils. A study of biological sulphur-oxidation affecting the nitrogen transformations in suspensions of activated sludge was therefore undertaken.

Boullanger and Dugardin (*Compt. rend.*, 1912, **155**, 327) attributed the favourable action of sulphur on soil to stimulation of the ammonifying bacteria and, to a small extent, of the nitrifying organisms. They also observed that there was no influence on nitrogen-fixing or denitrifying bacteria. Organisms decomposing complex nitrogenous substances into ammonia appeared, however, to be greatly favoured. Pitz (*J. Agric. Res.*, 1916, **5**, 771) studied the influence of sulphur on higher and lower forms of plant life and assigned the beneficial effects of sulphur to increase in the production of ammonia and decrease in the total number of organisms. Ames and Richmond (*Soil Sci.*, 1918, **5**, 320) in recording the role of sulphur-oxidation in relation to nitrogen transformations in acid and basic soils explained the depression that they observed in the activities of nitrifying organisms as being due to absence of bases like lime to neutralise the acid produced. Ammonium sulphate was formed by reaction with the ammonia of the soil and not by stimulation of ammonifiers. Shedd (*J. Agric. Res.*, 1919, **18**, 329) who studied the effect of sulphur-oxidation on the solubility of rock phosphate in soils observed, on the other hand, that nitrification proceeded even in presence of free sulphuric acid that tended to accumulate, the amount nitrified being 20 per cent. of the total nitrogen present. It may be gathered from the above that (*a*) there is no general agreement with regard to the mode of production and accumulation of ammonia, and (*b*) nitrification is completely inhibited in soils devoid of bases while in those containing some, the inhibition is noticeable in the later stages.

To study the effect of sulphur-oxidation in suspensions of activated sludge, with and without phosphate, on the various forms of nitrogen, experiments were designed and the products closely studied during a period of six weeks, while keeping the suspensions under constant aeration by compressed air. Samples were drawn daily from each series for the different determinations. In addition to study of changes in the different forms of nitrogen, observations on changes in (*a*) reaction, (*b*) counts on albumin agar (Waksman, *Soil Sci.*, 1916, **2**, 113) and (*c*) nature of colonies developing on raisin-agar were also made.

METHODS.

The details of experiment were the same as those described in an earlier communication (*J. Indian Inst. Sci.*, 1929, **12A**, 275) except that ammonia-free sterile air was used for aeration. Before drawing samples for analysis, aeration was stopped, the sludge allowed to settle, and the supernatant liquid drawn under aseptic conditions was used for plating and nitrogen estimations. Ammonia was estimated by the method of Melling (Chemists' Year Book, 1927, 639), 5 c.c. being used for each determination, and nitrates by the phenoldisulphonic acid method. Reaction was determined colorimetrically (Medalia, *J. Bact.*, 1920, **5**, 441 ; **7**, 589).

Before making the plates, trials were conducted on albumin agar to determine the dilution favourable to development of 150–200 colonies per plate after incubation for 7 days at 28° to 30°. A dilution of 1 in 100 was thus found to be the most efficient. Samples drawn under sterile conditions were accordingly diluted and plated in triplicate.

The total period of observation was divided into halves, of three weeks each, based on the nature of the different changes that were observed. The quantities of ammonia and nitrate, counts on albumin agar, and reaction at different stages have been represented in figures I, II, III and IV respectively.

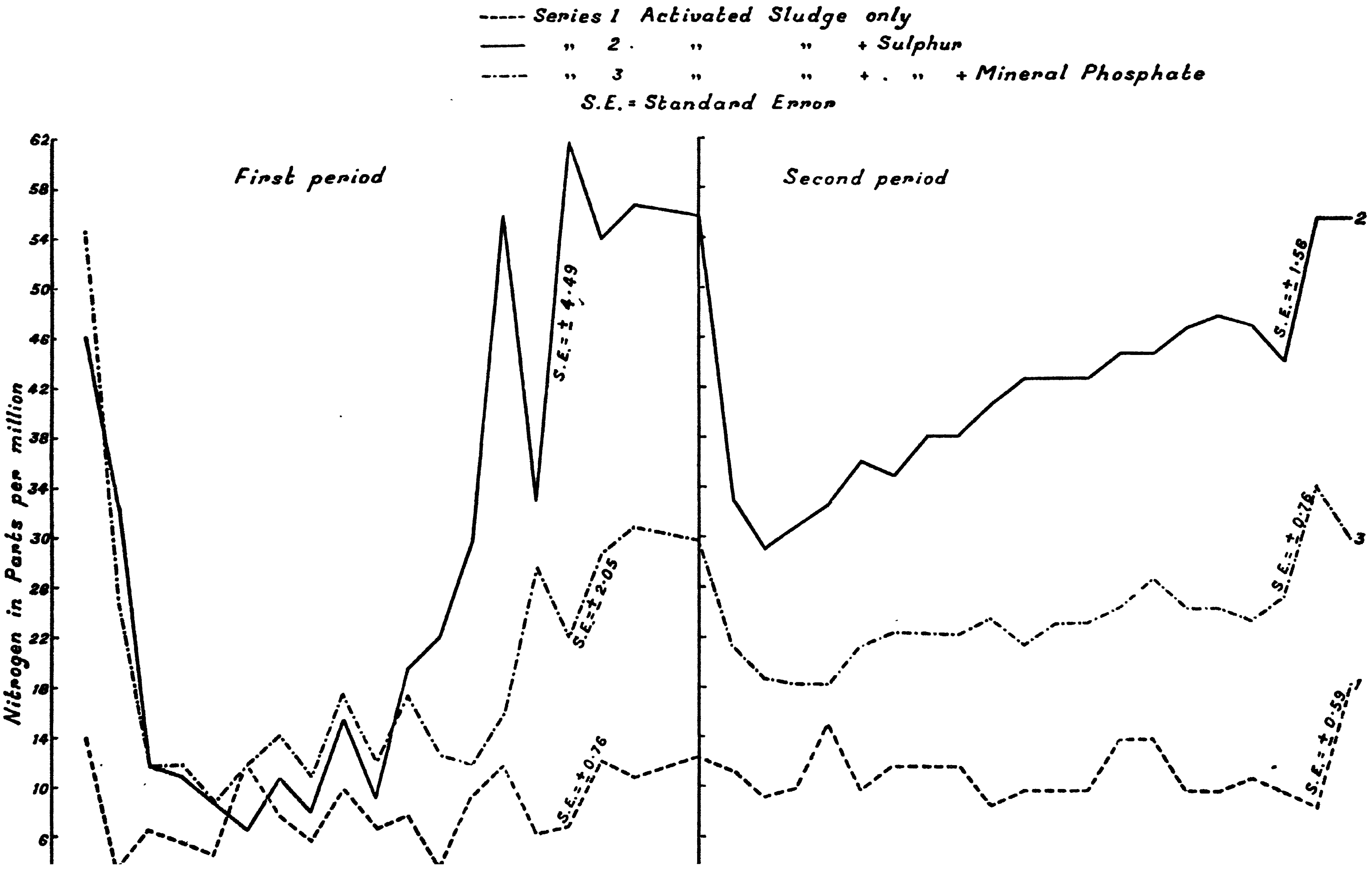
THE FIRST PERIOD.

Activated Sludge series.—The reduction in ammonia and nitrate observed initially might have been due to either (*a*) loss of the former during vigorous aeration and the latter through denitrification or (*b*) assimilation of both by micro-organisms and consequent transformation into complex forms.

It was observed that the air leaving the jars did not contain even traces of ammonia. On inoculating the effluent into a nutrient medium containing nitrate and incubating for 14 days at 30°, no gas-production was observed. A study of the bacterial counts for the same

Fig. 1

Ammoniacal Nitrogen



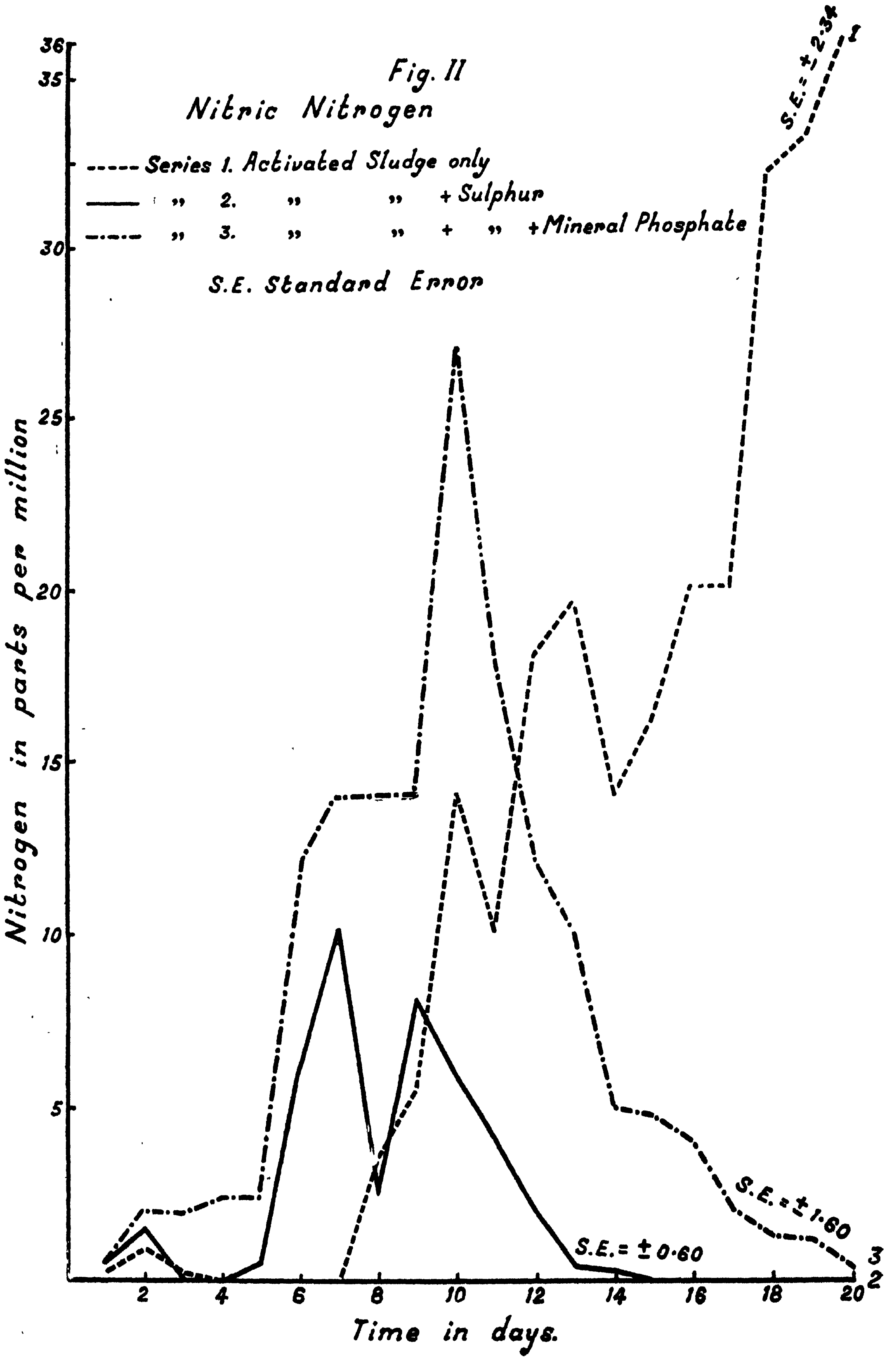
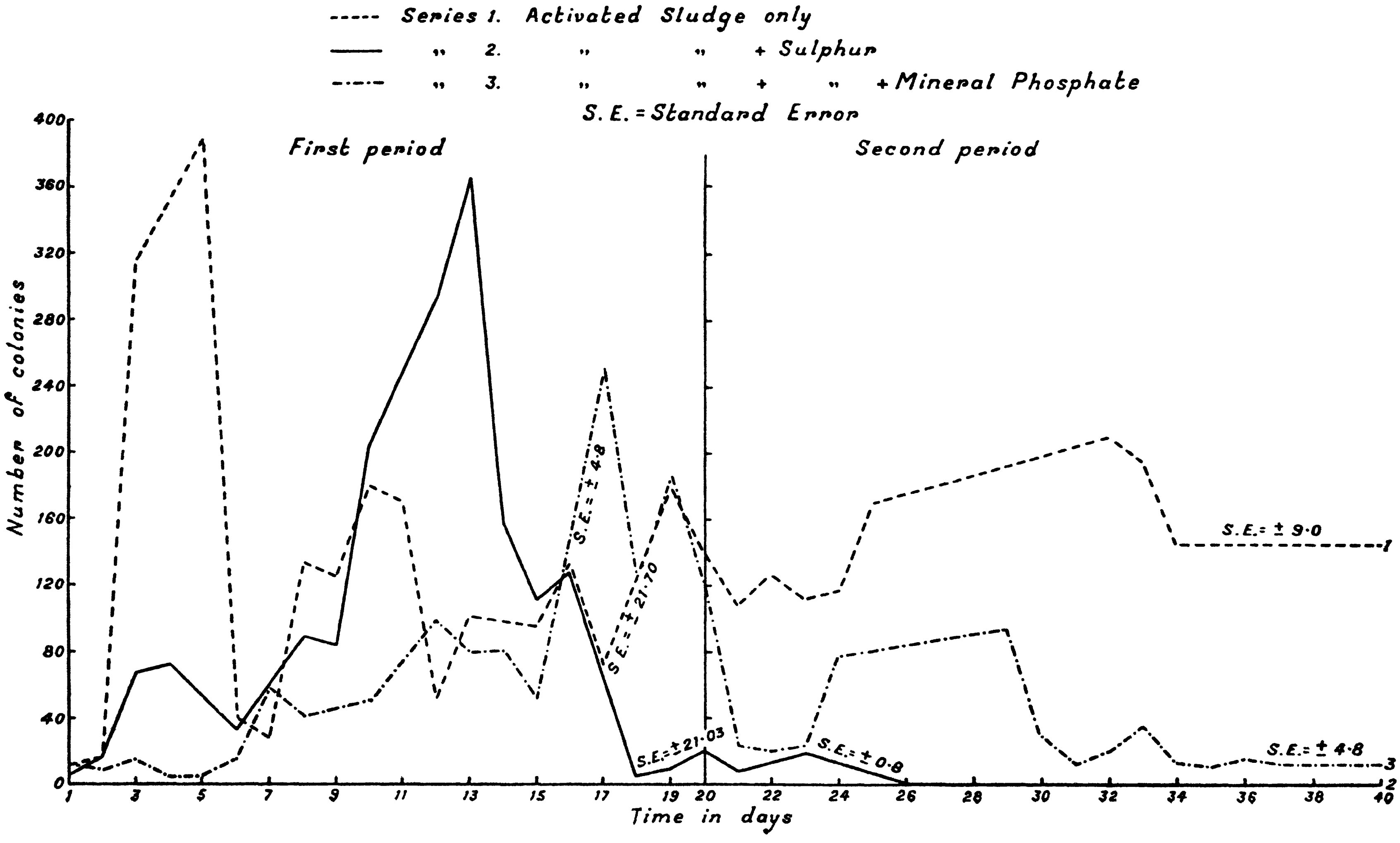


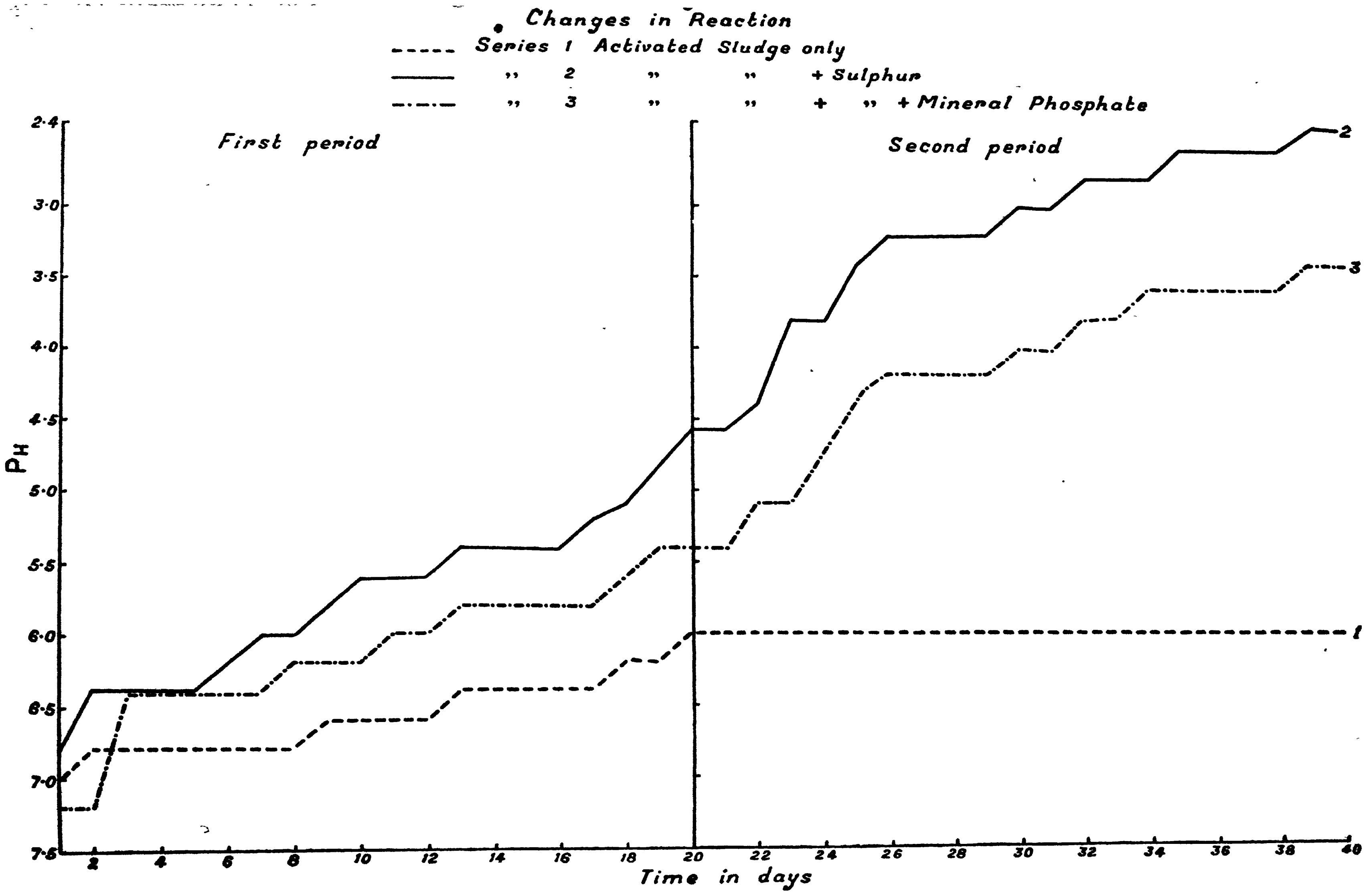
Fig. II

Nitric Nitrogen

Fig. III

Colonies on Egg albumin agar




Changes in Reaction
----- Series 1 Activated Sludge only
—— " 2 " " + Sulphur
-·-·- " 3 " " + " + Mineral Phosphate
First period
Second period
PH
2·4
3·0
3·5
4·0
4·5
5·0
5·5
6·0
6·5
7·0
7·6
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
Time in days
1
2
3

period, together with the observation that fungal colonies were then appearing in increasing numbers suggested, however, that ammonia and nitrate were being used up by the growing microflora.

After the tenth day, a steady production of ammonia was maintained. The increase in bacterial numbers at the same time suggested that (*a*) there was marked increase in the number of ammonifiers and (*b*) ammonia was being produced at a rate much too fast for its assimilation. The steady formation and accumulation of nitrates indicated that the conditions were favourable to the growth and functioning of nitrifying organisms.

Sulphur-activated sludge series.—There was rapid fall in the ammonia content during the first three days, and the corresponding diminution in bacterial numbers suggests that those organisms were not responsible for such a change. There was rapid production of ammonia from the eleventh to the eighteenth day, when the rate slackened. The development of intense acidity during the second three weeks was evidently responsible for the weakening in production of ammonia during that period.

As the reaction became more acid, nitrification appeared to have been hindered; the bacteria which increased in numbers up to the thirteenth day were probably responsible for the complete utilisation of even the small quantities of nitrates produced.

Simultaneously with the rapid production of ammonia, large numbers of fungi were observed on the raisin-agar medium, suggesting that those organisms were in some way associated with the production of ammonia from the albuminoid matter present in the sludge. It has been recorded by many workers, and recently by Thakur and Norris (*J. Indian Inst. Sci.*, 1928, **11A**, 149) that fungi are to a large extent responsible for the production of ammonia in the soil. Since it is also generally recognised that the microflora of activated sludge are essentially those of the soil, it appears probable that the organisms chiefly responsible for the production of ammonia in the present series were also fungi. The cultural characteristics of the fungi developing on raisin-agar were similar to those previously observed on wort-agar (*J. Indian Inst. Sci.*, 1929, **12A**, 275).

The foregoing observations are in conformity with those of Pitz (*loc. cit.*) who observed that sulphur-oxidation favoured the development of ammonifiers, but retarded, with the development of acidity, the growth of commoner bacterial types. They also suggest that the enhanced ammonification noticed by Boullanger and Dugardin (*loc. cit.*) during the oxidation of sulphur in the soil was due to increased activity of fungi that decomposed the complex organic matter of the soil.

With prolonged aeration and development of acid, the colour of the sludge changed to brown, as is generally observed with activated sludge under similar conditions.

Phosphate-sulphur series.—Nitrogen transformations in this series may be described as being intermediary to those observed in the two previous ones. Owing to the calcium carbonate in the rock phosphate partially neutralising the acid produced, and the dissolved phosphate acting as buffer, active nitrification in this series proceeded for nearly a week longer than in the sulphur series. With further development of acid, however, nitrification was hindered and ammonification favoured, as observed already in the sulphur series.

The limiting reaction for nitrification in the acid range lay between P_H 6·0 and 5·8 as observed by Ranganathan and Norris (*J. Indian Inst. Sci.*, 1927, **10A**, 114) in their studies on intensive nitrification in activated sludge. After that stage no further quantities of nitrate were produced. At P_H 5·4 they disappeared almost entirely having probably been utilised by the fungi which then developed in large numbers.

THE SECOND PERIOD.

In the activated sludge series the reaction, bacterial numbers and nitrate content remained almost unaltered. There was a slight but significant increase in ammonia.

In the sulphur series the reaction became steadily more acid, finally attaining P_H 2·4. Bacterial counts diminished steadily. Plates made after the twenty-sixth day did not carry even a single colony, thereby confirming the previous observation of Ramaswami Ayyar and Norris (*J. Indian Inst. Sci.*, 1929, **12A**, 277) that the commoner sewage organisms could not withstand the acidity. Nitrates were absent, having been entirely used at the end of the first fortnight, the reaction subsequently developed being unfavourable to nitrification. The ammonia content showed marked decrease in the initial stages, thereafter increasing steadily to the end.

The last observation is one of considerable interest. The sudden decrease in ammonia noticed between the twentieth and the twenty-second days could not have been due to loss in the gaseous form because none was detected in the air leaving the jars in which aeration was being conducted. As the counts made during the same period showed a marked decline in the number of bacteria, the latter could not have been responsible for the rapid decrease. Only the fungi were much in evidence at that stage and it is probable that they were responsible for the initial transformation of free and saline ammonia into less readily available forms.

The steady increase in ammonia after the twenty-second day could not have been due to the activity of the commoner ammonifying bacteria because the reaction was distinctly unfavourable to them. As the sulphur-oxidisers, then increasing, were found incapable of bringing about nitrogen transformations, it may be inferred that there were other agencies responsible for this appreciable increase in free and saline ammonia in the medium. Two possible causes were suggested; (*a*) sulphuric acid then developing in the medium flocculated the colloids and leached out the adsorbed ammonia, or (*b*) activity of the abundant fungi in the sludge.

To investigate the direct chemical action of sulphuric acid alone, experiments were designed wherein the action of fungi was suppressed by toluene. Ammonia was extracted after adding dilute sulphuric acid to give different H-ion concentrations and estimated. The results did not show any significant increases over controls without acid, indicating the absence of any direct action of sulphuric acid. It therefore appeared probable that the increase in free and saline ammonia observed in the later stages was also due to the activity of fungi.

In the phosphate-sulphur series, the changes taking place during the second period were of the same type as those of the sulphur series, though generally less intense. Probably owing to sulphur, the reaction became steadily acid in spite of the buffering action of the phosphate, the final reaction being P_H 3·4. The quantities of ammonia passing into the supernatant liquid were less than in the sulphur series. Most likely owing to the same causes as in the sulphur series the nitrates disappeared and the bacterial numbers decreased steadily, with development of acidity.

The conclusions reached in the foregoing paragraphs were confirmed by a statistic study of the different sets of data, those for reaction, ammonia and nitrates being recognised as characteristic of the respective treatments. The bacterial counts during the first period were not, however, significantly different from each other, though they accounted for the corresponding changes in ammonia and nitrate.

DISCUSSION.

Even though the nature and function of activated sludge are still imperfectly understood, yet it is now generally recognised that it (*a*) contains an intense mass of living organisms, which are essentially aerobic and which can bring about a variety of oxidation changes in air; and (*b*) conserves, in its mass, the major part of the nitrogen and the phosphate of the sewage in a readily available form (Richards, E. H., and Sawyer, G. C., *J. Soc. Chem. Ind.*, 1922, **41**, 62). Activated sludge cannot however be regarded as a form of purified sewage and its utility as an all round manure, except when fully dried, is marred by its tendency to putrefy, causing offensive odours and probably endangering public health (Whitehead, cited from Martin, The Activated Sludge Process, 1927, 180). Collection and marketing are rendered difficult because of its occurrence as a jelly, often containing over 97 per cent. of moisture which is not easily removed.

Seven years of vegetation experiments carried out at the Indian Institute of Science have shown that when the effluent from an activated sludge tank is utilised for irrigation undiluted, many of the plants show the effects of excessive supply of nitrogen. For this reason and perhaps others, some of the crops have been found susceptible to certain bacterial and fungal diseases. In view of the fact that such occurrences will discourage the extensive use of undiluted effluent in farm practice, it will be desirable to have a modification that will yield a weak effluent conserving all the plant nutrients in the sludge itself.

The investigations so far carried out on the biological oxidation of sulphur in suspensions of activated sludge (*J. Indian Inst. Sci.*, 1928, **11A**, 85; 1929, **12A**, 275; 1929, **12A**, 278) have shown that (*a*) with the progress in sulphur oxidation, there occurs a selective functioning of oxidising organisms, (*b*) the pathogenic and putrefactive organisms commonly found in the sewage sludge become extinct, (*c*) the oxidation of sulphur leads to gradual development of acidity and to the formation of a floc which settles readily on standing, (*d*) the effluent obtained after moderate aeration in presence of sulphur contains less nitrogen than the one from the activated sludge alone and (*e*) the sludge formed during sulphur oxidation conserves plant nutrients more effectively than activated sludge alone.

In addition, sulphur-oxidation has a bearing on dewatering activated sludge, since it has been recognised that sludge which has been treated with sulphuric acid parts readily with its water. Thus the Maclachlan process of concentrating sewage sludge is based on passing sulphur dioxide fumes mixed with steam into the sludge followed by pressing (*Eng. News Rec.*, 1923, 87, 947). At Milwaukee, preliminary treatment with sulphuric acid prior to pressing is practised (Martin, Activated Sludge Process, 1927, 345).

Further study of the phenomenon is required before the foregoing and the allied observations can find practical application. The quantities of sulphur and inorganic catalysts needed to provide active flocs under a variety of conditions and for different types of sewage have to be determined. The mode of application and different stages of treatment required to give a flocculum that will (*a*) have a uniform composition, (*b*) not settle heavily in the tanks and clog the pores of the diffusers and (*c*) be readily reactivated, have to be determined. Liming and other treatments necessary to neutralise the effects of the acid in the effluent on soil have also to be standardised. It is hoped that the foregoing and the allied problems will form subjects of subsequent communications.

SUMMARY.

The effect of sulphur-oxidation on ammonification and nitrification in suspensions of activated sludge is discussed.

In the presence of sulphur, nitrification was found to be completely inhibited, but ammonification, brought about largely by fungi, proceeded vigorously almost to the end.

Changes in the type and number of microflora, and in reaction were studied and correlated with other observations.

The possible bearing of the different observations on their application to the purification of sewage, with special reference to the conservation of plant nutrients in the sludge, is indicated.

The author takes this opportunity of thanking Prof. Roland V. Norris and Dr. V. Subrahmanyan for their many suggestive criticisms during the progress of this work.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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BIOCHEMISTRY OF TAN-LIQUOR FERMENTATION.

By P. D. Dalvi.

That vegetable tan-liquors undergo fermentation resulting in the destruction of their tanning properties, has been known from almost prehistoric times: but until recent years no systematic attempt was made to study the nature of the fermentation and seek means to stop it.

Van Tieghem (*Compt. rend.*, 1867, **65**, 1091) first observed that tan-liquors contain filamentous fungi, chief among which are *Aspergillus niger* and *Penicillium glaucum*, which hydrolyse tannic acid to gallic acid and glucose which have no tanning properties. Fernbach (*Compt. rend.*, 1901, **131**, 1214) observed that *Aspergillus niger* growing in Raulin's medium containing tannic acid in place of sugar develops an extra-cellular ferment, tannase, which brings about the hydrolysis of tannic acid in the same way as the living fungus. Knudson (*J. Biol. Chem.*, 1913, **14**, 159, 185) made a systematic study of the action of *Aspergillus niger* and *Penicillium glaucum* in tan-liquors and biological media, observing that the fungi (*a*) normally preferred sucrose to gallic acid and (*b*) did not develop tannase except in presence of tannic or gallic acid.

Rhind and Smith (*Biochem. J.*, 1922, **16**, 1) and later, Nicholson and Rhind (*Analyst*, 1924, **49**, 505) studied the application of tannase to the estimation of tannins. Nierenstein (*Biochem. J.*, 1922, **16**, 514) observed that the tannase developed by *Aspergillus luchuensis* hydrolysed tannic acid, but was without any action on other tannins. Freudenberg (*Z. physiol. Chem.*, 1926, **164**, 262) standardised conditions for the preparation of tannase and studied the action of this enzyme on methyl gallate. No systematic work has, however, been so far carried out on the action of tannase on tannic acid which is the chief constituent of tan-liquors. All previous attempts to check the growth of fungi without impairing the quality of tan-liquor proved unsuccessful.

The objects of the present investigation were to (*a*) isolate and identify the commoner microflora of indigenous tan-liquors, (*b*) prepare the enzyme tannase in an active form and study the factors influencing its action on tannic acid, and (*c*) study the efficacy of different preservatives in preventing the loss of tannic acid by fermentation.

MICROFLORA OF TAN-LIQUORS.

The organisms present in tan-liquors are probably those introduced by dust and by the hides and skins soaked in them. Very little is however known regarding their precise nature and mode of distribution beyond the observations of Asai (*J. Coll. Sci., Imp. Univ. Tokio*, 1918, **39**, 1) who noted the presence of growing yeasts and fungi. It was therefore considered desirable to determine the flora characteristic of tan-liquors from different sources.

Tan-liquors.—Specimens prepared from (1) Avaram bark (*Cassia auriculata*), (2) Divi-divi (*Cæsalpinia coriaria*) and (3) Myrobalans (*Terminalia chebula*) were obtained directly from the vegetable tan-vats in which hides and skins were soaking for several days. The liquors were yellowish brown and, except in a few rare cases, had superficial growths of fungi; they contained also fragments of hides and skins and large amounts of suspended matter which settled on standing and occupied about 1/16th of the total volume. Their barkometer strength (Sp. Gr.) varied between 1·02 and 1·06. The hydrogen-ion concentration of the liquors could not be determined by colorimetric methods as they were strongly coloured; it was therefore determined electrometrically and found to vary between P_H 3·0 and P_H 4·0.

The liquors were occasionally found to be ropy owing to profuse growth of certain types of bacteria. Such specimens were about 10 per cent. more viscous than water and generally free from surface growths of fungi; their tannic acid contents were invariably low.

Isolation of different organisms.—This was effected by three methods, (1) *dilution*, (2) *direct* and (3) *direct microscopic*. 1. Platings were made with the liquors diluted to 1 in 100 to 1 in 1,000 for bacteria, and 1 in 1,000 to 1 in 10,000 for fungi and yeasts. Incubations were at 25° for fungi and yeasts and at 37° for bacteria, the time being 3 days in all the cases. The platings were repeated to isolate the different organisms.

2. The method of Waksman (*Soil Sci.*, 1917, **3**, 565) was adopted chiefly to isolate and identify the fungi occurring vegetatively. Incubations were for 24 hours at 25°. 3. Following Conn (*N. Y. Agric. Exp. Sta. Tech. Bull.*, **64**, 1917 ref.) the liquors, after suitable dilution, were examined directly under the microscope. This method, though suitable for bacteria and yeasts, was not easily adaptable to fungal hyphæ which were abundant in tan-liquors.

Media used for isolation.—Czapeck's synthetic medium and wort-agar were found to be most suitable for the isolation of fungi and yeasts and nutrient agar for bacteria. The numbers of fungi varied from 180,000 to 400,000 per c.c. of liquor, invariably increasing with the age of the liquor. Counts of yeasts varied between 300,000 and 500,000 per c.c. and were generally greater in specimens collected on hot days (25–30°) than in cooler weather (20–25°). The bacterial numbers at no time were so large as those of fungi and yeasts, varying from 500 to 2,000 per c.c. The counts tended to increase with the progress of the fermentation.

IDENTIFICATION.

Seventeen different strains of fungi, three of yeasts and ten of bacteria were isolated, and most of them identified. The few that remained unidentified were found to be without any action on tannic acid and hence of no interest to the present investigation.

Fungi.—(1) *Aspergillus niger*, (2) *A. flavus*, (3) *A. spinulosum*, (4) *A. oryzæ*, (5) *A. candidus*, (6) *A. nidulans*, (7) *Penicillium glaucum*, (8) *P. brevicaule*, (9) *P. oxalicum*, (10) *P. camemberti*, (11) *P. claviforme*, (12) *P. granulatum*, (13) *P. X* (unidentified), (14) *Mucor racemosus*, (15) *M. mucedo*, (16) *M. rouxii*, (17) *Rhizopus nigricans*.

Bacteria.—Of the ten isolated, three were identified with (1) *B. acidi lactici*, (2) *B. aceti* and (3) *B. coli*. The cultural characteristics of the remaining seven organisms (Nos. 4–10) were studied.

Yeasts.—One was identified with *Saccharomyces apiculatus*. The physiological activities of the other two (Nos. 2 and 3) were studied in some detail. None of the yeasts was, however, found to have any action on tannic acid though Biddle and Kelley (*J. Amer. Chem. Soc.*, 1912, **34**, 914) observed that a certain yeast brought about a change in the optical rotation of tannic acid without hydrolysing it to gallic acid.

Cultural characteristics of organisms.—In order to study the response of the more prominent fungi to tannic acid, they were inoculated into media containing varying percentages, and the results tabulated below:—

Growths, /-/-/-/- = excellent; /-/-/- = very good; /-/- = good; /- = slight; — = no growth. Of the four that withstood tannic acid, at all concentrations, the most satisfactory growth was made by *Aspergillus niger*.

TABLE I.

Organism	Percentages of tannic acid			
	0·25	2	5	10
Aspergillus niger ...	/-/-/-/-	/-/-/-	/-/-	/-
,, *flavus* ...	/-/-/-/-	/-/-/-	/-	/-
Penicillium glaucum ...	/-/-/-/-	/-/-/-	/-	/-
,, *X* ...	/-/-/-/-	/-/-/-	/-	/-
,, *camemberti* ...	/-/-/-	/-/-	/-	—
,, *granulatum* ...	/-/-/-	/-	/-	—
,, *claviforme* ...	/-/-/-	/-	/-	—
Mucor rouxii ...	/-/-	/-	—	—

Some of the unidentified bacteria (Nos. 4–8) were compared for their ability to produce acid and gas in nutrient broth containing sugars, mannitol and dulcitol. The observations (Table II) were made after incubation for 48 hours at 37°.

TABLE II.

Number	Sucrose	Galactose	Mannitol	Dulcitol	Lactose	Dextrose	Laevulose
4	/-/-	—	/-/-	—	/-	/-/-	/-/-
5	/-/-	—	/-/-	—	/-	/-/-	/-/-
6	/-/-	/-	/-/-	—	/-	/-/-	/-/-
7	/-/-	/-/-	—	—	/ /-	/-/-	/
8	G	G	G	—	G	G	G

/— = slightly acid ; /—/— = acid ; G = acid and gas ;— = no acid and no gas.

Other morphological and physiological characteristics of the different organisms have been presented in Tables III and IV.

TABLE III.

Bacterium number	Ammonia production	Liquefaction of gelatin at 20°		Indol formation	Potato culture growth	Size in microns	
		24 hrs.	96 hrs.			Bacterium	Spore
4	/–	...	...	...	Good	1·0–1·1 by 4·5–4·7	1·0 to 1·1
5	/–	...	...	...	,,	0·6–0·7 by 2·0–2·1	0·6 to 0·7
6	/–	...	...	...	,,	0·6–0·7 by 1·6–1·8	0·6 to 0·8
7	—	/–	/–	—	good-bluish tint	1·0–1·2 by 2·0–2·2	0·4 to 0·5
8	/–	—	—	/–	Very good	0·4–0·5 (cocci).	...
9	—	—	—	—	,,	...	0·5 to 0·6
10	/–	—	—	/–	,,	...	Non-sporing

/– = reaction positive; — = reaction negative.

TABLE IV.

Action on Litmus Milk.

Reaction after	Bacterium number						
	4	5	6	7	8	9	10
24 Hours ...	—	—	—	—	/–	—	/–
48 Hours ...	—	—	—	—	/–/–	—	/–/–
...	...	slightly yellow	...	decoloration of litmus	...	...	...
72 Hours ...	—	—	—	—	/–/–	—	/–/–
...	slightly coagulated	slightly coagulated	slightly yellow	...	...	...	...

— = no change; /– = acid; /–/– = acid and gas.

The unidentified yeasts (2 and 3) were inoculated into media containing peptone (1 per cent.) and sodium chloride (0·5 per cent.)

together with one of the sugars or hexitols (2 per cent.) indicated in Table V.

TABLE V.

Number	Sucrose	Mannitol	Dulcitol	Galactose	Lactose	Dextrose	Lævulose
2	–	–	–	–	–	/-	/-
3	–	–	–	–	–	/-	/-

/– = fermentation ; — = no fermentation.

It may be seen from the foregoing tables that *Aspergillus niger*, *Penicillium glaucum* and *Penicillium X* developed well even in presence of 10 per cent. tannic acid. *Mucor Rouxii* grew fairly well in a solution containing 2·5 per cent. of tannic acid, but could not withstand higher concentrations. The remaining fungi grew only in solutions containing less than 2·5 per cent. of tannic acid. The yeasts isolated from tan-liquors fermented dextrose and lævulose, but did not ferment other sugars tried. Among the bacteria, some were nitrate reducers; one liquefied gelatin and four produced acid and gas from nutrient media containing sugars.

PREPARATION OF TANNASE.

Source of the enzyme.—It was noticed previously that *Aspergillus niger* flourished most vigorously even in presence of high concentrations of tannic acid. In view of the above and the observations of several previous workers that *Aspergillus niger* was the most common and the most active of the organisms found in tan-liquors, the enzyme tannase was prepared from that fungus for further investigation.

Choice of a medium for growing the fungus.—Several synthetic nutrient media including those of Czapeck and Raulin in which sugar had been replaced by tannic acid were tried. It was observed that although the fungus grew fairly well on most of them, the yields of the enzyme from such sources were not encouraging. Freudenberg's medium, in a slightly modified form, was then tried and found to be highly satisfactory.

Myrobalan powder (600 g.) was stirred with 4 litres of distilled water in a pail, the suspension boiled for 15 minutes, cooled and poured with the solid residue into a Winchester bottle. The supernatant liquid containing most of the soluble matter was syphoned and the sediment repeatedly extracted with boiling water until almost all the tanning material was removed. The extracts were combined, made up to 12 litres and the following added:— Ammonium sulphate, 300 g.; di-potassium phosphate, 9 g.; magnesium sulphate, 3 g.; and sodium chloride, 1 g. The reaction was adjusted to P_H 3.2–3.4 and the mixture sterilised.

Growth of the fungus.— The medium was placed in several 750 c. c. Erlenmeyer or flat-bottomed Pasteur flasks to the depth of about 3 cm. The flasks were plugged and steam-sterilised, the medium then being temporarily somewhat turbid owing to precipitation of magnesium phosphate. *Aspergillus niger* began growing in the medium, having been inoculated in the form of spores obtained from a previous growth on the same medium and incubated at 32–34°.

White specks of the fungus mycelium began forming on the surface of the medium. On the third day, when the growth was nearly complete, the fungus 'felt' was removed by means of a nickel wire. The growth was not allowed to proceed any further since it was observed that the fungus then began sporulating, with consequent loss of enzyme.

Preparation of enzyme powder.—The mass of mycelia was immersed for a few minutes in running water to remove adhering culture solution, squeezed free of water, pressed between thick folds of filter paper and then ground up with four times its weight of acetone. After 24 hours the extract was drained, the residue washed with a fresh quantity of acetone, and then extracted with ether, after which it was spread out in a thin layer for about two hours until the odour of solvent was no longer perceptible; the coarse powder remaining was dried over phosphorus pentoxide in a desiccator.

The preparation contained the enzyme tannase together with such constituents of the cells as were not soluble in acetone or ether; it contained 5·2 per cent. of nitrogen. The aqueous extract (*a*) was faintly opalescent and gave a feeble biuret reaction, and (*b*) did not give either Millon's or Hopkins and Cole reaction.

Extraction and concentration of the enzyme.—The dry mould powder (10 g.) prepared as above was agitated with a litre of distilled water and 10 c. c. of pure toluene in a glass-stoppered bottle maintained at 22° in an ice incubator for 24 hours, and shaken from time to time. The extract was then filtered through washed kieselguhr and concentrated by distillation under reduced pressure at a temperature not exceeding 40°.

It was not possible to concentrate the enzyme by freezing out the water since a large part of the enzyme was retained in the ice.

Purification.—The enzyme was precipitated from a concentrated aqueous solution with absolute alcohol free from gaseous impurities like acetylene. It was then redissolved in water, dialysed for 48 hours in collodion membranes prepared according to Farmer (*J. Biol. Chem.*, 1917, **32**, 447), and passed through a Berkefeld filter to free it from suspended impurities.

The purified enzyme did not give any of the commoner protein reactions. It was associated with a small amount of diastase, but no attempt was made to remove the latter by repeated precipitation with alcohol since it was observed that (*a*) such treatment tended to reduce the activity of tannase, and (*b*) the diastase did not interfere with the action of tannase on tannic acid.

Preservation.—The mould powder and the precipitated dry enzyme retained their activity almost indefinitely. The solution was not stable at high temperatures but maintained its activity for quite long periods at 0° in presence of toluene.

FACTORS INFLUENCING THE ACTION OF TANNASE.

To determine whether the enzyme was similar in behaviour to those obtained by Freudenberg and other workers, some preliminary trials were carried out.

Hydrolysis of methyl gallate.— The ester was prepared by the method of Homburg (*Monatsh.*, 1898, **19**, 594). A solution of the ester (0·271 g.) in 45 c. c. of conductivity water was treated with 5 c.c. of an aqueous extract of the enzyme and 1 c. c. of toluene, and the mixture placed in a thermostat at 33°. Aliquot parts of the reaction mixture were removed at intervals and titrated electrometrically against N/200 sodium hydroxide until P_H 7·16 — the reaction of a mixture of equivalent amounts of sodium hydroxide and gallic acid — was attained. From the quantities of alkali required at different stages, the corresponding amounts of gallic acid present in the medium were calculated. The results have been presented in Table VI.

The observations show that (*a*) the reaction became more and more acid as the concentration of gallic acid increased in the medium and (*b*) the hydrolysis did not follow the course of a monomolecular reaction. Similar results were obtained by Freudenberg and Vollbrecht (*Z. Physiol. Chem.*, 1922, **116**, 277).

TABLE VI.

Time in hours	p_H	Percentage hydrolysis	Free gallic acid, mg.
0	5·05	...	...
4	4·52	1·84	4·60
8	4·30	2·70	6·80
16	4·10	4·12	10·30
32	3·82	6·40	16·00
48	3·70	7·80	19·50
72	3·55	10·70	26·50

Influence of salts on the hydrolysis of methyl gallate.—The reaction mixture was composed of 40 c.c. of an aqueous solution containing 0·271 g. of the ester, 5 c.c. of an aqueous extract of the enzyme, 0·25 c.c. of a 0·1 *M* solution of a salt, 4·75 c.c. of conductivity water to make up the volume to 50 c.c. and 1 c.c. of toluene. The temperature of hydrolysis and the method of titration were the same as those described in the previous experiment. The results have been given in Table VII.

TABLE VII.

Time in hours	Sodium chloride		Potassium acid phosphate (KH_2PO_4)		Di-potassium phosphate (K_2HPO_4)	
	p_H	Hydrolysis per cent.	p_H	Hydrolysis per cent.	p_H	Hydrolysis per cent.
0	5·05	...	5·08	...	5·92	...
4	4·55	1·5	4·52	1·9	5·76	1·4
8	4·30	2·6	4·35	3·1	5·40	3·1
16	4·15	4·0	4·12	4·6	4·84	5·9
32	3·85	6·3	3·85	6·5	4·25	9·3
48	3·72	7·6	3·72	8·1	4·05	11·6
72	3·60	10·2	3·55	10·9	3·96	14·1

The hydrolysis was hastened by adding di-potassium phosphate (K_2HPO_4), while the other salts were without any effect. Variation of the concentration of sodium chloride was also without any appreciable effect.

The chemical mechanism of the hydrolysis of tannic acid into gallic acid is still not properly understood. In view of the above and the possibility of at least a part of the tannic acid undergoing change to products other than gallic acid, the action of tannase was followed, in the subsequent series of trials, by studying both decrease in tannic acid and corresponding increase in gallic acid.

Effect of substrate concentration.—Aqueous solutions of tannic acid of different concentrations (5·0, 2·0, 1·0 and 0·5 per cent.) were prepared. To 25 c. c. of each, an equal volume of enzyme solution was added and the mixtures maintained at 50° in a thermostat. Aliquot parts were examined at intervals for their tannic and gallic acid contents by the method of Gardner and Hodgson (*J.C.S.*, 1909, **95**, 1819). The results have been presented in Table VIII.

TABLE VIII.

Time in hours	Concentration of tannic acid in the reaction mixture							
	2·5 per cent.		1·0 per cent.		0·5 per cent.		0·25 per cent.	
	A	B	A	B	A	B	A	B
6	9·5	8·0	23·8	18·0	28·5	25·5	23·3	19·5
12	12·5	10·5	39·0	32·8	45·8	39·9	41·3	36·8
24	27·5	23·0	66·0	56·5	69·3	60·8	60·9	59·5
48	50·0	42·5	103·5	93·0	101·0	86·3	72·8	64·8
72	52·0	43·5	129·0	115·3	125·0	110·3	87·8	77·9
96	53·0	43·5	133·5	120·0	128·4	114·0	88·0	78·0

A = Tannic acid hydrolysed in mg. B = Gallic acid formed in mg.

The observations show that the activity of the enzyme increased with decrease in substrate concentration.

Influence of temperature on hydrolysis.—The reaction mixture which was composed of 25 c.c. of a 1·0 per cent. solution of tannic acid and an equal volume of enzyme solution was maintained at different temperatures and the progress of the hydrolysis followed (Table IX).

TABLE IX.

Time in hours	Temperature					
	30°		50°		60°	
	A	B	A	B	A	B
6	10·6	8·3	28·5	25·5	25·8	22·5
12	18·2	15·8	45·8	39·9	70·8	47·8
24	28·5	26·8	69·3	60·8	115·6	102·1
48	42·1	39·3	106·0	86·3	138·8	123·0
72	60·4	54·1	124·8	110·3	152·0	135·5
96	73·2	64·6	128·4	114·0	155·3	140·4

A = Tannic acid hydrolysed in mg. B = Gallic acid formed in mg.

The foregoing observations were checked by a further series of experiments in which tannic acid was estimated by the method of Lowenthal (*J. pr. Chem.*, 1863, 3, 150). The results are given in Table X.

TABLE X.

Temperature	Percentage of tannic acid hydrolysed after	
	20 hours	48 hours
21·5°	8·4	12·6
25·5	12·0	13·5
50·0	13·5	15·4
59·0	15·1	19·8
65·0	10·9	14·8

It may be inferred from the above tables that the optimum temperature for the hydrolysis is about 60°.

Concentration of enzyme and rate of hydrolysis.—To three flasks, each containing 0·25 g. of tannic acid dissolved in 45 c.c. of water, 2·0, 2·5 and 4·0 c.c. of the enzyme solution were added, the volume increased with water to 50 c.c., maintained at 50° and the quantities of tannic and gallic acids present at different stages determined (Table XI).

TABLE XI.

Time in hours	Volume of enzyme solution					
	2 c.c.		2·5 c.c.		4 c.c.	
	A	B	A	B	A	B
6	16·4	12·9	19·5	16·6	25·5	21·0
12	28·5	25·2	34·8	28·8	47·8	40·8
24	52·8	44·3	66·2	55·7	97·5	82·8
48	92·7	81.0	105·8	96·8	126·3	120·0
72	118·3	104·3				

A = Tannic acid hydrolysed in mg. B = Gallic acid formed in mg.

The rate of hydrolysis increases with the concentration of the enzyme, but is not directly proportional to it.

Effect of concentrating the enzyme.—This experiment was carried out to determine whether concentration of the enzyme, by distilling an aqueous extract under reduced pressure, tended to reduce its activity. One litre of enzyme solution was concentrated to 50 c. c. by distillation at 40°. To 25 c.c. lots of a solution (1 per cent.) of tannic acid, 25 c.c. of the dilute and 1·25 c.c. of the concentrated extracts respectively were added. After making up the volume, in the latter case to 50 c.c. by addition of water, the two sets of mixtures were maintained at 50° and the progress of hydrolysis studied (Table XII).

It may be inferred that the activity of the enzyme is not affected during the process of concentration.

Dialysis of the enzyme.—To determine whether the enzyme can be purified by dialysis, an aqueous extract was dialysed for 72 hours in collodion membranes against distilled water out of direct contact with air. The dialysate was collected and concentrated to about 20 c.c. by distilling under reduced pressure. The activities of the dialysed enzyme, and the concentrated dialysate as compared with that

TABLE XII.

Time in hours	Percentage of hydrolysis	
	Condition of enzyme	
	Dilute	Concentrated
8	2·5	3·2
16	5·5	5·7
24	8·1	9·3
48	16·2	17·3
72	23·7	25·7
96	32·1	34·0

of an equivalent amount of a fresh aqueous extract of the enzyme were determined in the manner described in the previous experiment (Table XIII).

TABLE XIII.

Time in hours	Percentage of hydrolysis		
	Condition of enzyme		
	Not dialysed	Dialysed	Concentrated dialysate
15	6·4	4·5	2·9
41	20·4	9·6	10·6

The observations show that the enzyme passed partially through the collodion membrane; it may therefore be inferred that it is not possible to purify the enzyme by continued dialysis.

Influence of salts.—The reaction mixtures were made up as follows :—45 c.c. of a 0·56 per cent. solution of tannic acid, 2 c.c. of enzyme solution, 1 c.c. of conductivity water and 2 c.c. of a 0·1 *M* solution of the salt; those tried were sodium chloride, diacid potassium phosphate (KH_2PO_4) and di-potassium phosphate (K_2HPO_4). The reaction mixtures were maintained at 50°; progress of the hydrolysis was as follows.

TABLE XIV.

Time in hours	No salt (control)		Di-potassium phosphate (K_2HPO_4)	
	A	B	A	B
6	15·1	12·3	19·5	16·1
12	25·5	23·3	30·8	27·0
24	42·3	38·3	48·5	43·6
48	65·4	57·8	85·6	66·6
72	80·7	72·0		
96	83·3	73·8		

A = Tannic acid hydrolysed in mg. B = Gallic acid formed in mg.

The results obtained for mixtures treated with sodium chloride and di-acid potassium phosphate have not been recorded as they were identical with those for the control.

The reaction was, however, accelerated by addition of di-potassium phosphate. The effect of the latter could not have been due to the presence of any active ion since the same ions were present in the solution of the di-acid phosphate, which was without any influence on the course of the reaction: it was probably due to a change in the H-ion concentration (*Vide* Table XVII).

The foregoing observations do not lend support to the theory of Euler and Josephson (*Z. physiol. Chem.*, 1927, **166**, 294) who state that addition of salts either hastens or retards the action of enzymes. A similar observation has also been made by Karmarkar and Patwardhan (*J. Indian Inst. Sci.*, 1930, **13A**, 163) who find that salts are without any action on the activity of vegetable amylases.

Influence of gallic acid concentration.—This experiment was conducted to determine whether the accumulation of gallic acid affected the progress of hydrolysis. To mixtures containing 40 c.c. of 1·25 per cent. tannic acid and 10 c.c. of enzyme solution, 0·05 and 0·1 g. respectively of gallic acid was added and the progress of the hydrolysis followed in the manner described already (Table XV).

TABLE XV.

Time in hours	Quantities of gallic acid added					
	None (control)		0·05 g.		0·1 g.	
	A	B	A	B	A	B
6			16·3	12·2	27·0	22·5
12	45·6	38·0	33·2	27·0	48·2	39·0
24	90·0	72·0	64·5	56·5	73·5	58·5
48	141·8	120·9	120·0	105·0	108·9	90·0
72	178·0	151·5	134·5	120·5	130·5	114·5
96	211·5	183·9	136·0	121·0	133·0	117·6

A = Tannic acid hydrolysed in mg. B = Gallic acid formed in mg.

The results show that gallic acid retards the hydrolysis of tannic acid, particularly in the later stages.

In some later trials it was observed that 0·25 and 0·5 g. of gallic acid proved so inhibitive that hydrolysis proceeded no further than 7·4 and 2·2 per cent., respectively, at the end of 72 hours.

Effect of light on the action of tannase.—The reaction mixture in each flask consisted of 25 c.c. of 1 per cent. tannic acid and 25 c.c. of enzyme solution. One set of flasks was exposed to diffused light and the other placed in darkness. The rates of hydrolysis in the two sets of cases were followed (Table XVI).

TABLE XVI.

Time in hours	Percentage of hydrolysis	
	In light	In darkness
4	18·0	18·20
8	32·5	23·80
16	47·2	47·50
28	65·5	65·40

The observations show that conditions of light or shade have no effect on the activity of the enzyme.

Influence of H-ion concentration.—Most of the commoner buffer mixtures were found to be unsuitable for the present series of trials because they either turned the media turbid or interfered with the estimation of tannic and gallic acids. McIlvaine's phosphate and citric acid buffer was, however, found to be free from these defects up to P_H 5·15 and was used in the following series of trials. Preliminary experiments having shown that the buffer mixture had no direct action on either tannic acid or the enzyme, reaction mixtures containing, in each case, 10 c.c. of 2·75 per cent. tannic acid, 40 c.c. of buffer mixture of a particular reaction, 2·5 c.c. of enzyme solution and 2·5 c.c. of water were placed in a thermostat at 50° and the progress of hydrolysis followed in the usual way (Table XVII).

TABLE XVII.

Time in hours	Reactions											
	Control, unbuffered P_H 3·58		P_H 2·3		P_H 2·9		P_H 3·64		P_H 4·25		P_H 5·5	
	A	B	A	B	A	B	A	B	A	B	A	B
6	9·5	8·2	4·7	4·0	10·0	8·7	10·0	8·6	12.5	10·8	22·0	19·5
12	18·5	16·4	10·0	9·1	18·0	15·8	18·0	15·8	24·5	23·1	46·0	43·3
24	36·5	32·4	30·4	27·2	41·4	36·6	37·4	33·4	65·0	58·3	80·0	70·9
48	68·5	60·7	30·4	27·3	52·5	46·3	64·5	57·8	109·0	96·7	142·0	131·8
72	100·0	88·6	...	...	53·0	46·4	92·4	87·1	124·0	109·9	216·0	192·0
96	130·5	115·6	...	...	...	...	117·8	100·2	182·0	161·4	250·5	221·5
120	160·5	142·3	...	...	...	...	142·0	122·6	217.5	194·5	...	...

A = Tannic acid hydrolysed in mg. **B = Gallic acid formed in mg.**

It may be inferred from the above that a highly acid reaction inhibits the action of the enzyme. A nearly neutral reaction, on the other hand, favours hydrolysis to such an extent that at the end of 96 hours, nearly all the tannic acid is destroyed.

PRESERVATION OF TAN-LIQUORS.

Under tropical conditions, vegetable tan-liquors lose considerable amounts of tannic acid by fermentation, and consequently deteriorate. In some tanneries the loss may amount to as much as 30 per cent. of the total tannic acid in the liquor. So there is great need to devise means to check or arrest the progress of the fermentation.

Most of the commoner fungicides such as salts of heavy metals, phenols and phenolic acids are unsuitable for the preservation of tan-liquors because some of them precipitate tannic acid, many by staining affect the quality of the leather produced, and all prove deadly to the bacteria and the yeasts which are useful to the tanning process. Only substances like the volatile antiseptics which will keep down the growth of fungi without becoming completely miscible with the tan-liquor can be safely tried as preservatives.

In the present series of trials, the efficacy of minute quantities of kerosene, thymol, camphor, carbon disulphide and formaldehyde in checking the development of tannase by *A. niger*, *A. luchuensis* and *P. glaucum* in solutions of tannic acid were studied. A number of flasks, each containing 25 c.c. of tannic acid of a particular concentration were sterilised in the autoclave. After cooling, each flask was first treated with a minute quantity of one of the antiseptics and then sown with the spores of one of the fungi. The flasks were incubated at 30° and their contents examined, at two stages, for growth of fungi, tannic acid lost and gallic acid formed.

TABLE XVIII.

Organism	Preservative	Final growth	Time in days			
			6		16	
			A	B	A	B
At the start, tannic acid = 447 mg. ; gallic acid = 52 mg.						
P. glaucum	None	/-/-	Not determined	Not determined	224	Not determined
,, ,,	Camphor, 0·1 g.	/-	,,	,,	7	nil

At the start, tannic acid = 466 mg. ; gallic acid = 67 mg.

A. niger	None	/-/-	Not determined	Not determined	319	263
,, ,,	Camphor, 0·1 g.	/-	,,	,,	44	Not determined
,, ,,	,, 0·2 g.	/-	nil	nil	nil	nil
,, ,,	Carbon disulphide 0·1 c.c.	/-/-	Not determined	Not determined	291	278
,, ,,	Formalin, 0·05 c.c.	/-/-	274	173	312	254
,, ,,	,, 0·1 c.c.	/-/-	248	163	285	209
,, ,,	,, 0·15 c.c.	/-/-	131	60	259	211
A. luchuensis	None	/-/-	Not determined	Not determined	115	81
,, ,,	Carbon disulphide 0·1 c.c.	/-/-	,,	,,	126	93

At the start, tannic acid = 606 mg.; gallic acid = 5 mg.

A. niger	Camphor, 0·05 g.	/-	340	283	447	391
,, ,,	,, 0·1 g.	/-	nil	nil	53	nil
,, ,,	,, 0·2 g.	nil	nil	nil	21	14
,, ,,	,, 0·4 g.	nil	nil	nil	16	15
,, ,,	Thymol, 0·05 g.	/-	nil	nil	54	42
,, ,,	,, 0·1 g.	/-	21	8	46	33
,, ,,	,, 0·15 g.	nil	16	8	32	23
,, ,,	,, 0·2 g.	nil	11	5	26	19
,, ,,	Kerosene, 0·05 c.c.	/-/-	254	217	452	396
,, ,,	,, 0·1 ,,	/-/-	144	132	446	400
,, ,,	,, 0·2 ,,	/-/-	69	46	441	391
,, ,,	,, 0·4 ,,	/-/-	16	Not determined	414	364

A = Tannic acid lost in mg.; B = Gallic acid formed in mg.
/- = slight growth; /-/- = Good growth.

The foregoing observations show that carbon disulphide, formalin and kerosene were not effective in checking either the growth of fungi or the loss of tannic acid. Thymol and camphor proved highly efficient even in minute quantities.

In addition to the above, the study of the factors influencing the action of tannase has already shown that hydrolysis of tannic acid is checked by either adding gallic acid, which is a product of the enzyme action, or adjusting the reaction to about P_H 2.0. It may not, perhaps, be practicable to collect and concentrate gallic acid though considerable amounts of it pass into tannery waste: but it should be possible to develop an easy method of adjusting the reaction of the liquors so that the action of tannase can always be held in check.

Investigations carried out by workers in this laboratory and elsewhere have shown that sulphur, even in minute quantities, is highly efficacious in checking the growth of fungi and maintaining, through the agency of the sulphur bacteria, a reaction corresponding to about P_H 2.0. The sulphur-oxidising organisms are present in the soil and are doubtless carried into tan-liquors by dust or soil adhering to tan barks. It therefore appears possible that addition of minute quantities of sulphur to the tan-liquors will help not only to check the growth of fungi but also lead to spontaneous development of the reaction required to retard the action of tannase.

Further work should be carried out under conditions prevailing in the tanneries, and with liquors from various sources before any one particular method of preservation can be considered to be efficient. Careful study of the direct and the indirect effects of the proposed treatment on the quality of leather produced should also be made before recommending its use by tanners.

SUMMARY.

The more important micro-organisms present in vegetable tan-liquors were isolated and identified. Fungi were found in great abundance both vegetatively and in the form of spores. Several of the organisms were tested for their action on tannic acid and it was observed that *Aspergillus niger*, *Penicillium glaucum*, and an unidentified fungus, *P. X* grew even on 10 per cent. tannic acid.

A preparation of tannase was obtained from the mycelia of *A. niger* growing on myrobalan decoction and its properties studied.

The optimum temperature for the hydrolysis of tannic acid by tannase was about 60°. A reaction corresponding to P_H 2.3 retarded the action of the enzyme, while one which was less acid (P_H 5.15) was highly favourable. The rate of hydrolysis (*a*) increased with the concentration of the enzyme, but decreased with that of the substrate, (*b*) was retarded by increasing amounts of gallic acid, (*c*) was not influenced by either neutral or acid salts and (*d*) was not affected by conditions of light and shade.

A preliminary study showed that minute quantities of thymol or camphor helped to check the growth of fungi on tannic acid.

A few of the possible methods of preventing the fermentation of tan-liquors have been discussed.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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NOTE ON THE ESTIMATION OF TANNIC AND GALLIC ACIDS.

By P. D. Dalvi.

Since 1799, numerous methods have been suggested for the estimation of tannic and gallic acids, but most of them are highly erroneous as admitted by the authors. The methods in common use may be classified under (1) Oxidation, (2) Adsorption, (3) Precipitation by metallic salts, (4) Precipitation by alkaloids, and (5) Iodination.

(1) *Oxidation.*—The methods of Lowenthal (*J. pr. Chem.*, 1863, **3**, 150) and Thompson (*Chem. Zentr.*, 1902, **2**, 1395) were tried for the estimation of tannins in tan-liquors; the former was found to be highly tedious and the latter did not give consistent results.

(2) *Adsorption.*—These methods, though commonly adopted in tanneries, were found to be erroneous. A similar observation has been made by Dreaper (*Chem. News*, 1904, **90**, 111).

(3) *Precipitation by metallic salts.*—Most of the methods were found to be unsatisfactory. The method of Dreaper (*loc. cit.*) was found to be reliable only when the concentration of tannic acid was high.

(4) *Precipitation by alkaloids.*—Rather low values for pure tannic acid were obtained by the strychnine method of Trotman (*Analyst*, 1906, **31**, 53). The cinchonine method of Ostermayer (*Chem. News*, **40**, 181) was also found to be unsatisfactory for the same reason.

(5) *Iodination.*—The method of Ferdinand (*Chem. Zentr.*, 1900, **1**, 1107) when tried for the estimation of pure tannic acid, did not yield consistent results. Boudet's modification (*Bull. Soc. Chim.*, 1906, **35**, 760) of the above method was also found to be defective because the reaction was not complete at the end of 2 hours as suggested by the author. Tables I and II give the amounts of iodine ($N/100$) taken up at different intervals, by 5 c.c. each of a 0·225 per cent. solution of tannic acid and a 0·0955 per cent. solution of gallic acid respectively.

The method of Gardner and Hodgson (*J.C.S.*, 1909, **95**, 1819) was next tried. Fairly accurate and concordant results were obtained for tannic and gallic acids, at different concentrations, both singly and mixed. In adopting the above method, it was found advantageous to

TABLE I.

Time in hours	0	2	4	6	8	11
Iodine, c.c. *N*/100 absorbed ...	18·7	22·1	22·6	23·0	23·3	23·8

TABLE II.

Time in hours	0	12	17	42	90
Iodine, c.c. *N*/100 absorbed ...	18·5	24·0	26·9	28·3	29·6

precipitate tannic acid with a mixture of gelatin and acid sodium chloride as suggested by Lowenthal (*loc. cit.*) rather than with gelatin alone.

Minute quantities of iodine were found to be adsorbed by the gelatin remaining in solution after the removal of tannic acid. This defect was overcome by carrying out a blank with gelatin alone and applying the necessary correction.

Aqueous solutions (o·1 per cent.) of tannic and gallic acids were mixed in different proportions and estimated by the modified method of Gardner and Hodgson (*loc. cit.*). The results which have been given in Table III show that accurate and concordant results can thus be obtained.

TABLE III.

Mg., actually present, of		Iodine (*N*/10) required for the mixture	Iodine (*N*/10) required for gallic acid only	Mg., as calculated, of	
Tannic acid	Gallic acid			Tannic acid	Gallic acid
10	...	4·7	...	10·0	0·0
8	2	4·9	1·1	8·0	2·1
6	4	5·0	2·2	6·0	4·1
5	5	5·1	2·7	5·1	5·0
2	8	5·2	4·2	2·0	7·9
0	10	5·3	...	0·0	10·0

In the course of the foregoing series of determinations, it was also observed that the reaction of the mixture became more acid as the concentration of gallic acid increased. With a view to ascertaining whether, by measuring the reaction, the proportions in which the two acids occur can be determined, tannic and gallic acids (0·4 per cent. each) were mixed in different proportions and the hydrogen-ion concentrations of the mixtures determined electrometrically (Table IV).

TABLE IV.

Percentages			Percentages		
Tannic acid	Gallic acid	P_H	Tannic acid	Gallic acid	P_H
100	0	3·7	60	40	3·26
95	5	3·58	55	45	3·23
90	10	3·55	50	50	3·20
85	15	3·48	45	55	3·19
80	20	3·40	40	60	3·15
75	25	3·36	35	65	3·10
70	30	3·32	30	70	3·08
65	35	3·28	...	...	...

As may be seen from the above, the differences in reaction are not so distinct as to warrant an extensive application of the method, particularly to vegetable extracts which contain large amounts of buffering materials.

SUMMARY.

A number of methods were tried for the estimation of tannic and gallic acids both singly and together. Among them, the method of Gardner and Hodgson (*J.C.S.*, 1909, **95**, 1819) with a slight modification was found to be the most reliable.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

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